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APPLICATION NUMBER: ***60/511,745***
FILING DATE: ***October 16, 2003***
PCT/AU04/001429

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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR § 1.53(c).

For: **IMMUNOMODULATING COMPOSITIONS AND USES THEREFOR**

Name of Sole Inventor: Steven John Ralph
Residence Address: 18 Barrier Reef Drive, Mermaid Waters, Queensland, 4218, Australia

Enclosed are:

(X) Specification in 57 pages.

(X) 1 sheet of drawings.

(X) A check in the amount of $80 to cover the filing fee is enclosed.

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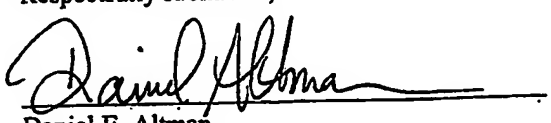
(X) Please send correspondence to:

Daniel E. Altman
Knobbe, Martens, Olson & Bear, LLP
2040 Main Street, 14th Floor
Irvine, CA 92614

Respectfully submitted,



Daniel E. Altman
Registration No. 34,115
Customer No. 20,995
(949) 760-0404

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Knobbe Martens Olson & Bear LLP
Intellectual Property Law

2040 Main Street
Fourteenth Floor
Irvine, CA 92614
Tel 949-760-0404
Fax 949-760-9502
www.kmob.com

Daniel E. Altman

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For	:	IMMUNOMODULATING COMPOSITIONS AND USES THEREFOR
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IMMUNOMODULATING COMPOSITIONS AND USES THEREFOR

FIELD OF THE INVENTION

[0001] THIS INVENTION relates generally to immunomodulating compositions. More particularly, the present invention is directed to compositions comprising an antigen and a lectin-interactive agent, which are useful for stimulating and prolonging host immune cell responses. The compositions of the present invention are particularly useful in the treatment and/or prophylaxis of a range of conditions including pathogenic infections, autoimmune diseases, transplant rejection, graft *versus* host disease, allergies, inflammatory disease, as well as cancers and tumours.

[0002] Bibliographic details of certain publications referred to by author in this specification are collected at the end of the description.

BACKGROUND OF THE INVENTION

[0003] This invention relates generally to immunomodulating compositions. More particularly, the present invention is directed to compositions comprising an antigen and a lectin-interactive agent, which are useful for stimulating and prolonging host immune cell responses. The compositions of the present invention are particularly useful in the treatment and/or prophylaxis of a range of conditions including pathogenic infections, autoimmune diseases, transplant rejection, graft *versus* host disease, allergies, inflammatory disease, as well as cancers and tumours.

[0004] According to one theory, much of the physiologic decline of immune responses or homeostasis, is due to programmed death of lymphocytes resulting from the loss of survival signals. As antigens are eliminated, the clones of lymphocytes that were activated by the antigen are deprived of the essential survival stimuli and die by apoptosis. Survival stimuli for lymphocytes function mainly by inducing the expression of anti-apoptotic proteins.

[0005] Examples of animal lectins include the galectins (reviewed in Rabinovich *et al.*, 2002, Trends Immunol. 23:313-320; Rabinovich *et al.*, J Leuk Biol 2002, 71: 741-752) or LGALS1 (lectin, galactoside-binding, soluble Blaser C. et al., *Euro. J Immunol*, 1998, 28: 2311-2319). The galectins, as a family of galactoside binding proteins have potent immunoregulatory activity (reviewed in Rabinovich et al., *J Leuk Biol.*, 2002, 71: 741-752). In particular, galactin-1 is a negative regulator of T-cell responses, inducing

apoptosis of T cells (Perillo, NL et al., *Nature*, 1995, 378(6558):736-739). Galectin-1 is also secreted by activated T cells thereby acting as a self regulator of T cell activation inhibiting antigen-induced proliferation of T cells (Blaser, C et al., as above).

[0006] In work leading up to the present invention, the inventor unexpectedly discovered that lactulose significantly improves the efficacy of raising and prolonging an immune response to a selected antigen . Not wishing to be bound by any one particular theory or mode of operation, these surprising results are believed to be based at least in part on the ability of a lectin-interactive agent such as lactulose competing with the glycoprotein receptors on the surface of T cells for binding to lectin, thereby inhibiting lectin-induced glycoprotein receptor clustering on the surface of T cells and preventing or attenuating the inhibition of T cell activation.

SUMMARY OF THE INVENTION

[0007] In one aspect, the present invention provides compositions for modulating an immune response to a target antigen. Generally, these compositions comprise a lectin-interactive agent and an immune-modulating agent selected from an antigen that corresponds to at least a portion of the target antigen and an immune effector cell that is specific for the target antigen.

[0008] The target antigen is typically associated with a disease or condition of interest. In some embodiments, the target antigen is produced by a pathogenic organism or a cancer. In these embodiments, the composition is especially useful for stimulating or otherwise enhancing an immune response to the target antigen. In other embodiments, the target antigen is associated with an unwanted immune response including, for example, transplant rejection, graft *versus* host disease, allergies, parasitic diseases, inflammatory diseases and autoimmune diseases. In these embodiments, the composition is especially useful for inducing a tolerogenic response including the induction of an anergic response, and the suppression of a future or existing immune response, to the target antigen.

[0009] The antigen that corresponds to at least a portion of the target antigen may be in soluble form. Alternatively, it may be presented by an antigen-presenting cell. For example, the antigen-presenting cell may be a professional or facultative antigen-presenting cell. In certain embodiments, these antigen-presenting cells stimulate an immune response. In other embodiments, they induce a tolerogenic response. In still other embodiments, the antigen-presenting cell is a cell to which an immune response is required (e.g., tumour cell) and which has been optionally modified to enhance its antigen-presenting functions. In some embodiments of this type, the cell is modified by culturing the cell in the presence of a type II interferon (IFN) and optionally at least one type I IFN for a time and under conditions sufficient to enhance the antigen-presenting function of the cell and washing the cell to remove the IFN. In other embodiments of this type, the cell is modified by introducing a construct into the cell from which one or more IFNs selected from a type II IFN and a type I IFN can be expressed. In some embodiments, the antigen-presenting cell is an allogeneic antigen-presenting cell or cell line that shares major and/or minor histocompatibility antigens to a recipient (also referred to herein as a 'generic' antigen-presenting cell or cell line).

[0010] Exemplary antigen-specific immune effector cells that may be used in concert with the lectin-interactive agent include antigen-specific T lymphocytes, including cytolytic T lymphocytes and helper T lymphocytes, T regulatory cells and B lymphocytes.

[0011] Suitably, the lectin to which the agent binds is a galectin, especially selected from galectin-1, galectin-3 and galectin-9. In some embodiments, the lectin-interactive agent is a carbohydrate or carbohydrate containing molecule including, but not restricted to: disaccharides non-limiting examples of which include, lactose, lactulose, lactosucrose, methyl β-lactoside, D-galactose, 4-O-β-D-galactopyranosyl-D-mannopyranoside, 3-O-β-D-galactopyranosyl-D-arabinose, 2'-O-methyllactose, lacto-N-biose, N-acetyllactosamine, and thiodigalactopyranoside; larger saccharides such as molecules comprising polylactosamine; as well as synthetic inhibitors such as thiodigalactoside and glycopolymers. Advantageously, the carbohydrate or carbohydrate containing molecule is non-metabolisable in the host to which the composition is administered. In certain embodiments, the lectin-interactive agent is lactulose.

[0012] In some embodiments, the composition further comprises one or more immunoregulatory molecules selected from co-stimulatory molecules (e.g., B7-1, B7-2, B7-3, ICAM-1 and ICAM-2), cytokines (interferons, granulocyte/macrophage-colony stimulating factor (GM-CSF), interleukin-10 and tumour necrosis factor α (TNF-α)) and co-inhibitory molecules (e.g., OX-2, programmed death-1 ligand (PD-1L)). Such molecules may be provided in soluble form. Alternatively, in cellular embodiments of the present invention, they may be produced intracellularly from a suitable expression construct or vector.

[0013] Another aspect of the present invention provides methods for modulating an immune response in a subject. These methods generally comprise administering to the subject a composition as broadly described above. The active components of the composition may be administered sequentially, separately or simultaneously. In certain embodiments, the immune response is a T-cell mediated response. Advantageously, these methods are useful for the treatment or prophylaxis of a disease or condition associated with the presence or aberrant expression of a target antigen in a subject. . In certain embodiments, the disease or condition is treated or prevented by using a composition that stimulates or otherwise enhances an immune response to a target antigen. In these embodiments, the composition may comprise a soluble antigen that corresponds to at least a portion of the target antigen or an immune-stimulating cell (e.g., antigen-presenting cell

or an immune effector cell such as an antigen-primed T lymphocyte or B lymphocyte) that stimulates an immune response to the target antigen. Suitably, the disease or condition is selected from a pathogenic infection, a disease characterised by immunodeficiency or a cancer.

[0014] In other embodiments, the disease or condition is treated or prevented by using a composition that elicits a tolerogenic response to a target antigen. In these embodiments, the composition may comprise an immune-attenuating cell (e.g., an antigen-presenting cell or T regulatory lymphocyte) that induces tolerance or otherwise attenuates an immune response to the target antigen. Suitably, the disease or condition is selected from transplant rejection, graft *versus* host disease, allergies, parasitic diseases, inflammatory diseases and autoimmune diseases.

[0015] In a further aspect, the invention contemplates the use of a lectin-interactive agent and an immune-modulating agent selected from an antigen that corresponds to at least a portion of the target antigen and an immune effector cell that is specific for the target antigen in the manufacture of a medicament for modulating an immune response to the target antigen.

[0016] In still another aspect, the invention resides in the use of a lectin-interactive agent and an immune-modulating agent selected from an antigen that corresponds to at least a portion of the target antigen and an immune effector cell that is specific for the target antigen agent in the manufacture of a medicament for treating or preventing a disease or condition associated with the presence or aberrant expression of the target antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figure 1 is a graphical representation showing the effects of six weekly injections of a vaccine comprising B16F10-B7.1high treated with IFN gamma and IFN beta alone or in combination with addition of 20 mg/ml solution of lactulose at 1:1 ratio to the cell suspension. The graph shows there was a marked improvement in the efficacy of the vaccine given to treated mice when lactulose was included (100% of the mice survived) versus the group given the vaccine alone, which all succumbed except for 4 out of 25 mice.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

[0018] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

[0019] The articles "*a*" and "*an*" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0020] The term "*about*" is used herein to refer to conditions (*e.g.*, amounts, concentrations, time etc) that vary by as much as 30%, preferably by as much as 20%, and more preferably by as much as 10% to a specified condition.

[0021] The term "*analogue*" refers to a molecule substantially similar in function to a reference molecule or to a biologically active fragment thereof.

[0022] The term "*anergy*" as used herein refers to a suppressed response, or a state of non-responsiveness, to a specified antigen or group of antigens by an immune system. For example, T lymphocytes and B lymphocytes are anergic when they cannot respond to their specific antigen under optimal conditions of stimulation.

[0023] By "*antigen*" is meant all, or part of, a protein, peptide, or other molecule or macromolecule capable of eliciting an immune response in a vertebrate animal, especially a mammal. Such antigens are also reactive with antibodies from animals immunised with that protein, peptide, or other molecule or macromolecule.

[0024] By "*antigen-binding molecule*" is meant a molecule that has binding affinity for a target antigen. It will be understood that this term extends to immunoglobulins, immunoglobulin fragments and non-immunoglobulin derived protein frameworks that exhibit antigen-binding activity.

[0025] By "*autologous*" is meant something (*e.g.*, cells, tissues etc) derived from the same organism.

[0026] The term "*allogeneic*" as used herein refers to cells, tissues, organisms etc that are of different genetic constitution.

[0027] By "*biologically active fragment*" is meant a fragment of a full-length parent polypeptide which fragment retains an activity of the parent polypeptide. As used herein, the term "*biologically active fragment*" includes deletion mutants and small peptides, for example of at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous amino acids, which comprise an activity of the parent polypeptide. Peptides of this type may be obtained through the application of standard recombinant nucleic acid techniques or synthesised using conventional liquid or solid phase synthesis techniques. For example, reference may be made to solution synthesis or solid phase synthesis as described, for example, in Chapter 9 entitled "*Peptide Synthesis*" by Atherton and Shephard which is included in a publication entitled "*Synthetic Vaccines*" edited by Nicholson and published by Blackwell Scientific Publications. Alternatively, peptides can be produced by digestion of a polypeptide of the invention with proteinases such as endoLys-C, endoArg-C, endoGlu-C and staphylococcus V8-protease. The digested fragments can be purified by, for example, high performance liquid chromatographic (HPLC) techniques.

[0028] As used herein, a "*cellular composition*", "*cellular vaccine*" or "*cellular immunogen*" refers to a composition comprising at least one cell population, which is optionally inactivated, as an active ingredient.

[0029] As used herein, the term "*cis-acting sequence*" or "*cis-regulatory region*" or similar term shall be taken to mean any sequence of nucleotides which is derived from an expressible genetic sequence wherein the expression of the genetic sequence is regulated, at least in part, by the sequence of nucleotides. Those skilled in the art will be aware that a *cis*-regulatory region may be capable of activating, silencing, enhancing, repressing or otherwise altering the level of expression and/or cell-type-specificity and/or developmental specificity of any structural gene sequence.

[0030] Throughout this specification, unless the context requires otherwise, the words "*comprise*", "*comprises*" and "*comprising*" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

[0031] By "*corresponds to*" or "*corresponding to*" is meant a polynucleotide (a) having a nucleotide sequence that is substantially identical or complementary to all or a

portion of a reference polynucleotide sequence or (b) encoding an amino acid sequence identical to an amino acid sequence in a peptide or protein. This phrase also includes within its scope a peptide or polypeptide having an amino acid sequence that is substantially identical to a sequence of amino acids in a reference peptide or protein.

[0032] As used herein, "*culturing*", "*culture*" and the like refer to the set of procedures used *in vitro* where a population of cells (or a single cell) is incubated under conditions which have been shown to support the growth or maintenance of the cells *in vitro*. The art recognises a wide number of formats, media, temperature ranges, gas concentrations etc. which need to be defined in a culture system. The parameters will vary based on the format selected and the specific needs of the individual who practices the methods herein disclosed. However, it is recognised that the determination of culture parameters is routine in nature.

[0033] By "*effective amount*", in the context of modulating an immune response or treating or preventing a disease or condition, is meant the administration of that amount of composition to an individual in need thereof, either in a single dose or as part of a series, that is effective for that modulation, treatment or prevention. The effective amount will vary depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

[0034] By "*expression vector*" is meant any autonomous genetic element capable of directing the synthesis of a protein encoded by the vector. Such expression vectors are known by practitioners in the art.

[0035] The term "*gene*" is used in its broadest context to include both a genomic DNA region corresponding to the gene as well as a cDNA sequence corresponding to exons or a recombinant molecule engineered to encode a functional form of a product.

[0036] By "*derivative*" is meant a polypeptide that has been derived from the basic sequence by modification, for example by conjugation or complexing with other chemical moieties or by post-translational modification techniques as would be understood in the art. The term "*derivative*" also includes within its scope alterations that have been made to a parent sequence including additions, or deletions that provide for functionally equivalent molecules.

[0037] To enhance immune response ("*immunoenhancement*"), as is well-known in the art, means to increase the animal's capacity to respond to foreign or disease-specific antigens (*e.g.*, cancer antigens) *i.e.*, those cells primed to attack such antigens are increased in number, activity, and ability to detect and destroy the those antigens. Strength of immune response is measured by standard tests including: direct measurement of peripheral blood lymphocytes by means known to the art; natural killer cell cytotoxicity assays (see, *e.g.*, Provinciali M. *et al* (1992, *J. Immunol. Meth.* **155**: 19-24), cell proliferation assays (see, *e.g.*, Vollenweider, I. And Groseurth, P. J. (1992, *J. Immunol. Meth.* **149**: 133-135), immunoassays of immune cells and subsets (see, *e.g.*, Loeffler, D. A., *et al.* (1992, *Cytom.* **13**: 169-174); Rivoltini, L., *et al.* (1992, *Can. Immunol. Immunother.* **34**: 241-251); or skin tests for cell-mediated immunity (see, *e.g.*, Chang, A. E. et al (1993, *Cancer Res.* **53**: 1043-1050). Any statistically significant increase in strength of immune response as measured by the foregoing tests is considered "*enhanced immune response*" "*immunoenhancement*" or "*immunopotentiation*" as used herein. Enhanced immune response is also indicated by physical manifestations such as fever and inflammation, as well as healing of systemic and local infections, and reduction of symptoms in disease, *i.e.*, decrease in tumour size, alleviation of symptoms of a disease or condition including, but not restricted to, leprosy, tuberculosis, malaria, naphthous ulcers, herpetic and papillomatous warts, gingivitis, artherosclerosis, the concomitants of AIDS such as Kaposi's sarcoma, bronchial infections, and the like. Such physical manifestations also define "*enhanced immune response*" "*immunoenhancement*" or "*immunopotentiation*" as used herein.

[0038] By "*greatly increased levels*" or "*high levels*" in the context of molecular expression is meant expression of a molecule at levels that are 10-fold, more preferably 50-fold, more preferably 100-fold and more preferably 200-fold above a reference level. For example, B16High cells as used herein, express greatly increased levels of a B7 molecule on their surface, which levels are 10-fold, more preferably 50-fold, more preferably 100-fold and more preferably 200-fold above wild-type B16 cells.

[0039] "*Hybridisation*" is used herein to denote the pairing of complementary nucleotide sequences to produce a DNA-DNA hybrid or a DNA-RNA hybrid. Complementary base sequences are those sequences that are related by the base-pairing rules. In DNA, A pairs with T and C pairs with G. In RNA U pairs with A and C pairs with G. In this regard, the terms "match" and "mismatch" as used herein refer to the

hybridisation potential of paired nucleotides in complementary nucleic acid strands. Matched nucleotides hybridise efficiently, such as the classical A-T and G-C base pair mentioned above. Mismatches are other combinations of nucleotides that do not hybridise efficiently.

[0040] Reference herein to "*immunodeficient*" includes reference to any condition in which there is a deficiency in the production of humoral and/or cell-mediated immunity.

[0041] Reference herein to "*immuno-interactive*" includes reference to any interaction, reaction, or other form of association between molecules and in particular where one of the molecules is, or mimics, a component of the immune system.

[0042] "*Inactivation*" of a cell is used herein to indicate that the cell has been rendered incapable of cell division to form progeny. The cell may nonetheless be capable of response to stimulus, or biosynthesis and/or secretion of cell products such as cytokines. Methods of inactivation are known in the art. Preferred methods of inactivation are treatment with toxins such as mitomycin C, or irradiation. Cells that have been fixed or permeabilised and are incapable of division are also examples of inactivated cells.

[0043] By "*isolated*" is meant material that is substantially or essentially free from components that normally accompany it in its native state.

[0044] A composition is "*immunogenic*" if it is capable of either: a) generating an immune response against an antigen (*e.g.*, a tumour antigen) in a naive individual; or b) reconstituting, boosting, or maintaining an immune response in an individual beyond what would occur if the compound or composition was not administered. A composition is immunogenic if it is capable of attaining either of these criteria when administered in single or multiple doses.

[0045] By "*modulating*" is meant increasing or decreasing, either directly or indirectly, the level and/or functional activity of a target molecule. For example, an agent may indirectly modulate the said level/activity by interacting with a molecule other than the target molecule. In this regard, indirect modulation of a gene encoding a target polypeptide includes within its scope modulation of the expression of a first nucleic acid molecule, wherein an expression product of the first nucleic acid molecule modulates the expression of a nucleic acid molecule encoding the target polypeptide. In certain embodiments, "modulation" or "modulating" means that a desired/selected response is more efficient (e.g., at least 10%, 20%, 30%, 40%, 50%, 60% or more), more rapid (e.g., at

least 10%, 20%, 30%, 40%, 50%, 60% or more), greater in magnitude (e.g., at least 10%, 20%, 30%, 40%, 50%, 60% or more), and/or more easily induced (e.g., at least 10%, 20%, 30%, 40%, 50%, 60% or more) than if the antigen had been used alone.

[0046] The term "*5' non-coding region*" is used herein in its broadest context to include all nucleotide sequences which are derived from the upstream region of an expressible gene, other than those sequences which encode amino acid residues which comprise the polypeptide product of said gene, wherein 5' non-coding region confers or activates or otherwise facilitates, at least in part, expression of the gene.

[0047] By "*non-metabolisable*," "*not metabolised*" and the like is meant a lectin-interactive agent that is not converted in a living organism (e.g., by catabolism or anabolism) to an agent that is incapable of interacting with the lectin.

[0048] By "*obtained from*" is meant that a sample such as, for example, a nucleic acid extract or polypeptide extract is isolated from, or derived from, a particular source of the host. For example, the extract may be obtained from a tissue or a biological fluid isolated directly from the host.

[0049] The term "*oligonucleotide*" as used herein refers to a polymer composed of a multiplicity of nucleotide units (deoxyribonucleotides or ribonucleotides, or related structural variants or synthetic analogues thereof) linked via phosphodiester bonds (or related structural variants or synthetic analogues thereof). Thus, while the term "oligonucleotide" typically refers to a nucleotide polymer in which the nucleotides and linkages between them are naturally occurring, it will be understood that the term also includes within its scope various analogues including, but not restricted to, peptide nucleic acids (PNAs), phosphoramidates, phosphorothioates, methyl phosphonates, 2-O-methyl ribonucleic acids, and the like. The exact size of the molecule may vary depending on the particular application. An oligonucleotide is typically rather short in length, generally from about 10 to 30 nucleotides, but the term can refer to molecules of any length, although the term "polynucleotide" or "nucleic acid" is typically used for large oligonucleotides.

[0050] The term "*operably connected*" or "*operably linked*" as used herein means placing a structural gene under the regulatory control of a promoter, which then controls the transcription and optionally translation of the gene. In the construction of heterologous promoter/structural gene combinations, it is generally preferred to position the genetic sequence or promoter at a distance from the gene transcription start site that is approximately the same as the distance between that genetic sequence or promoter and the

gene it controls in its natural setting; i.e., the gene from which the genetic sequence or promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting; i.e., the genes from which it is derived.

[0051] The terms "*patient*," "*subject*," "*host*" or "*individual*" used interchangeably herein, refer to any subject, particularly a vertebrate subject, and even more particularly a mammalian subject, for whom therapy or prophylaxis is desired. Suitable vertebrate animals that fall within the scope of the invention include, but are not restricted to, any member of the subphylum Chordata including primates, rodents (e.g., mice rats, guinea pigs), lagomorphs (e.g., rabbits, hares), bovines (e.g., cattle), ovines (e.g., sheep), caprines (e.g., goats), porcines (e.g., pigs), equines (e.g., horses), canines (e.g., dogs), felines (e.g., cats), avians (e.g., chickens, turkeys, ducks, geese, companion birds such as canaries, budgerigars etc), marine mammals (e.g., dolphins, whales), reptiles (snakes, frogs, lizards etc), and fish. A preferred subject is a human in need of treatment or prophylaxis for a condition or disease, which is associated with the presence or aberrant expression of an antigen of interest. However, it will be understood that the aforementioned terms do not imply that symptoms are present.

[0052] By "*pharmaceutically-acceptable carrier*" is meant a solid or liquid filler, diluent or encapsulating substance that may be safely used in topical or systemic administration.

[0053] The term "*pharmaceutically compatible salt*" as used herein refers to a salt which is toxicologically safe for human and animal administration. This salt may be selected from a group including hydrochlorides, hydrobromides, hydroiodides, sulphates, bisulphates, nitrates, citrates, tartrates, bitartrates, phosphates, malates, maleates, napsylates, fumarates, succinates, acetates, terephthalates, pamoates and pectinates.

[0054] The term "*polynucleotide*" or "*nucleic acid*" as used herein designates mRNA, RNA, cRNA, cDNA or DNA. The term typically refers to oligonucleotides greater than 30 nucleotides in length.

[0055] The terms "*polynucleotide variant*" and "*variant*" refer to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence or polynucleotides that hybridise with a reference sequence under stringent conditions that are

defined hereinafter. These terms also encompasses polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide. The terms "*polynucleotide variant*" and "*variant*" also include naturally occurring allelic variants.

[0056] "*Polypeptide*", "*peptide*" and "*protein*" are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally occurring amino acid, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers.

[0057] The term "*polypeptide variant*" refers to polypeptides which vary from a reference polypeptide by the addition, deletion or substitution of at least one amino acid. It is well understood in the art that some amino acids may be changed to others with broadly similar properties without changing the nature of the activity of the polypeptide. Accordingly, polypeptide variants as used herein encompass polypeptides that have similar activities to a parent polypeptide selected from an interferon alpha, an interferon beta, an interferon gamma, a B7-1 molecule and a B7-2 molecule. Preferred variant polypeptides comprise conservative amino acid substitutions. Exemplary conservative substitutions in a polypeptide may be made according to the following table:

TABLE A

Original Residue	*Exemplary Substitutions*
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn

Original Residue	*Exemplary Substitutions*
Glu	Asp
Gly	Pro
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile,
Phe	Met, Leu, Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu

[0058] Substantial changes in function are made by selecting substitutions that are less conservative than those shown in TABLE A. Other replacements would be non-conservative substitutions and relatively fewer of these may be tolerated. Generally, the substitutions which are likely to produce the greatest changes in a polypeptide's properties are those in which (a) a hydrophilic residue (e.g., Ser or Asn) is substituted for, or by, a hydrophobic residue (e.g., Ala, Leu, Ile, Phe or Val); (b) a cysteine or proline is substituted for, or by, any other residue; (c) a residue having an electropositive side chain (e.g., Arg, His or Lys) is substituted for, or by, an electronegative residue (e.g., Glu or Asp) or (d) a residue having a smaller side chain (e.g., Ala, Ser) or no side chain (e.g., Gly) is substituted for, or by, one having a bulky side chain (e.g., Phe or Trp).

[0059] Reference herein to a "*promoter*" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e., upstream activating sequences, enhancers and silencers) which alter gene expression in response to

developmental and/or environmental stimuli, or in a tissue-specific or cell-type-specific manner. A promoter is usually, but not necessarily, positioned upstream or 5', of a structural gene, the expression of which it regulates. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene. Preferred promoters according to the invention may contain additional copies of one or more specific regulatory elements to further enhance expression in a cell, and/or to alter the timing of expression of a structural gene to which it is operably connected.

[0060] The term "*recombinant polynucleotide*" as used herein refers to a polynucleotide formed *in vitro* by the manipulation of nucleic acid into a form not normally found in nature. For example, the recombinant polynucleotide may be in the form of an expression vector. Generally, such expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleotide sequence.

[0061] By "*recombinant polypeptide*" is meant a polypeptide made using recombinant techniques, *i.e.*, through the expression of a recombinant polynucleotide.

[0062] As used herein "*stimulating*" an immune or immunological response refers to administration of a composition that initiates, boosts, or maintains the capacity for the host's immune system to react to a target substance, such as a foreign molecule, an allogeneic cell, or a tumour cell, at a level higher than would otherwise occur. Stimulating a "primary" immune response refers herein to eliciting specific immune reactivity in a subject in which previous reactivity was not detected; for example, due to lack of exposure to the target antigen, refractoriness to the target, or immune suppression. Stimulating a "secondary" response refers to the reinitiation, boosting, or maintenance of reactivity in a subject in which previous reactivity was detected; for example, due to natural immunity, spontaneous immunisation, or treatment using one or several compositions or procedures.

[0063] By "*vector*" is meant a nucleic acid molecule, preferably a DNA molecule derived, for example, from a plasmid, bacteriophage, or plant virus, into which a nucleic acid sequence may be inserted or cloned. A vector preferably contains one or more unique restriction sites and may be capable of autonomous replication in a defined host cell including a target cell or tissue or a progenitor cell or tissue thereof, or be integrable with the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector may be an autonomously replicating vector, *i.e.*, a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a linear or closed circular plasmid, an extrachromosomal element, a

minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. A vector system may comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell, or a transposon. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector may also include a selection marker such as an antibiotic resistance gene that can be used for selection of suitable transformants. Examples of such resistance genes are well known to those of skill in the art.

2. Compositions

[0064] The present invention stems at least in part from the discovery that a subject's immune response to an antigen is enhanced by the introduction of a lectin-interactive agent. Not wishing to be bound by any one particular theory or mode of operation, it is proposed that the lectin-interactive agent acts as an adjuvant by competing with glycoprotein receptors on the surface of T cells for binding to lectins, which inhibits lectin-induced glycoprotein receptor clustering and prevents or attenuates the inhibition of T cell activation. This results in the prolongation of T cell activation and the concomitant enhanced duration of immunity or tolerance to an antigen of interest. Accordingly, the present invention provides in one aspect a composition for modulating an immune response to a target antigen, comprising a lectin-interactive agent and an immune-modulating agent selected from an antigen that corresponds to at least a portion of the target antigen and an immune effector cell that is specific for the target antigen.

2.1 Lectin-interactive agents

[0065] The lectin-interactive agent includes any molecule or compound that directly or indirectly binds or physically associates with a lectin The binding or association may involve the formation of an induced magnetic field or paramagnetic field, covalent bond formation, an ionic interaction such as, for example, occur in an ionic lattice, a hydrogen bond or alternatively, a van der Waals interaction such as, for example, a dipole-dipole interaction, dipole-induced-dipole interaction, induced-dipole-induced-dipole interaction or a repulsive interaction or any combination of the above forces of attraction. In certain embodiments, the lectin is a galectin, which is suitably selected from galectin-1, galectin-3 and galectin-9. The lectin is suitably expressed by an organism illustrative examples of

which include bacteria, antamoeba, protozoans (e.g., malaria), insects, gastropods, plants or animals. In certain embodiments, the lectin is an animal lectin which is suitably selected from the group including, but not limited to calnexin, M-type lectins, L-lectins, P-lectins, C-lectins, galactoside-binding lectins, I-type lectins or R-lectins. Desirably, the lectin is galectin which is especially selected from galectin-1, galectin-3 and galectin-9.

[0066] The lectin-interactive agent is suitably selected from the group consisting of polynucleotides, polypeptides, antibodies, lipids or carbohydrates. In certain advantageous embodiments, the lectin-interactive agent is in soluble form.

[0067] In some embodiments, the lectin-interactive agent is a carbohydrate or carbohydrate-containing molecule (e.g., glycoproteins). Illustrative examples of carbohydrates include, but are not limited to: monosaccharide, disaccharides (e.g., lactose, lactulose, lactosucrose, methyl β-lactoside, D-galactose, 4-O-β-D-galactopyranosyl-D-mannopyranoside, 3-O-β-D-galactopyranosyl-D-arabinose, 2'-O-methyllactose, lacto-N-biose, N-acetyllactosamine, and thiodigalactopyranoside); larger saccharides (e.g., polylactosamine, polylactosamine-carrying glycopeptides, modified plant pectin polysaccharide); as well as synthetic inhibitors (e.g., thiodigalactoside, glycopeptides especially those containing lactose or galactose [Glinsky *et al.*, 1996, *Cancer Research* 56:5319-5324; Naidenko *et al.* 2000, *Glycobiology* **10**:abstract 60], N-acetyllactosamine derivatives such as those described in International Publication WO 02/057284, modified polysaccharides disclosed in U.S. Patent Application Publication Nos. 2002/0107222 and 2003/0013681, starburst dendrimers [André *et al.* 1999, *Glycobiology* **11**: 1253-1262] and glycopolymers [Pohl *et al.*, 1999, *Synthesis* 1515-1519;]. In certain embodiments, the carbohydrate or carbohydrate-containing molecule is not metabolised in the host to which the composition is administered. Illustrative examples of this type include lactulose, methyl 2-acetamido-2-deoxy-4-*O*-(3-[3-carboxypropan- amido]-3-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside, methyl 2-acetamido-2-deoxy-4-*O*-(3-[{Z}-3-carboxy-propenamido]-3-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside, methyl 2-acetamido-2-deoxy-4-*O*-(3-benzamido-3-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside, methyl 2-acetamido-2-deoxy-4-*O*-(3-[2- carboxybenzamido]-3-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside, methyl 2-acetamido-2-deoxy-4-*O*-(3-[4-methoxy-2,3,5,6-tetrafluorobenzamido]-3-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside, methyl 2-acetamido-2-deoxy-4-*O*-(3-[2-carboxy-3,4,5,6-tetrafluoro-benzamido]-3-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside, methyl 2-acetamido-2-

deoxy-4-*O*-(3-methane-sulfonamido-3-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside, methyl 2-acetamido-2-deoxy-4-*O*-(3-[4- nitrobenzenesulfonamido] -3-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside, methyl 2-acetamido-2-deoxy-4-*O*-(3-phenylaminocarbonylamino-3-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside, methyl 2-acetamido2-deoxy-4-*O*-(2-aminoacetamido-3-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside, methyl 2-acetamido2-deoxy-4-*O*-(3-[{2S}-2-amino-3-carboxy-propanamido]-3-deoxy-β-D-galactopyranosyl)-β-D-glucopyranoside. Suitably, the lectin-interactive agent is lactulose or synthetic or semi-synthetic analogue thereof.

2.2 Antigens

[0068] Target antigens useful in the present invention can be any type of biological molecule including, for example, simple intermediary metabolites, sugars, lipids, and hormones as well as macromolecules such as complex carbohydrates, phospholipids, nucleic acids and peptides. Target antigens may be selected from endogenous antigens produced by a host or exogenous antigens that are foreign to the host. Suitable endogenous antigens include, but are not restricted to, self-antigens that are targets of autoimmune responses as well as cancer or tumour antigens. Illustrative examples of self antigens useful in the treatment or prevention of autoimmune disorders include, but not limited to, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, ostecarthritis, psoriasic arthritis), multiple sclerosis, myasthenia gravis, systemic lupus erythematosis, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, including keratoconjunctivitis sicca secondary to Sjögren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing haemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anaemia, pure red cell anaemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves ophthalmopathy, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis. Other autoantigens include those derived from nucleosomes for the treatment of systemic lupus erythematosus (e.g., GenBank Accession No. D28394; Bruggen et al., 1996, *Ann.*

Med. Interne (Paris), 147:485-489) and from the 44,000 Da peptide component of ocular tissue cross-reactive with *O. volvulus* antigen (McKeclmie *et al.*, 1993, *Ann Trop. Med. Parasitol.* 87:649-652). Thus, illustrative autoantigens antigens that can be used in the compositions and methods of the present invention include, but are not limited to, at least a portion of a lupus autoantigen, Smith, Ro, La, U1-RNP, fibrillin (scleroderma), pancreatic β cell antigens, GAD65 (diabetes related), insulin, myelin basic protein, myelin proteolipid protein, histones, PLP, collagen, glucose-6-phosphate isomerase, citrullinated proteins and peptides, thyroid antigens, thyroglobulin, thyroid-stimulating hormone (TSH) receptor, various tRNA synthetases, components of the acetyl choline receptor (AchR), MOG, proteinase-3, myeloperoxidase, epidermal cadherin, acetyl choline receptor, platelet antigens, nucleic acids, nucleic acid:protein complexes, joint antigens, antigens of the nervous system, salivary gland proteins, skin antigens, kidney antigens, heart antigens, lung antigens, eye antigens, erythrocyte antigens, liver antigens and stomach antigens.

[0069] Non-limiting examples of cancer or tumour antigens include antigens from a cancer or tumour selected from ABL1 protooncogene, AIDS Related Cancers, Acoustic Neuroma, Acute Lymphocytic Leukaemia, Acute Myeloid Leukaemia, Adenocystic carcinoma, Adrenocortical Cancer, Agnogenic myeloid metaplasia, Alopecia, Alveolar soft-part sarcoma, Anal cancer, Angiosarcoma, Aplastic Anaemia, Astrocytoma, Ataxia-telangiectasia, Basal Cell Carcinoma (Skin), Bladder Cancer, Bone Cancers, Bowel cancer, Brain Stem Glioma, Brain and CNS Tumours, Breast Cancer, CNS tumours, Carcinoid Tumours, Cervical Cancer, Childhood Brain Tumours, Childhood Cancer, Childhood Leukaemia, Childhood Soft Tissue Sarcoma, Chondrosarcoma, Choriocarcinoma, Chronic Lymphocytic Leukaemia, Chronic Myeloid Leukaemia, Colorectal Cancers, Cutaneous T-Cell Lymphoma, Dermatofibrosarcoma-protuberans, Desmoplastic-Small-Round-Cell-Tumour, Ductal Carcinoma, Endocrine Cancers, Endometrial Cancer, Ependymoma, Esophageal Cancer, Ewing's Sarcoma, Extra-Hepatic Bile Duct Cancer, Eye Cancer, Eye: Melanoma, Retinoblastoma, Fallopian Tube cancer, Fanconi Anaemia, Fibrosarcoma, Gall Bladder Cancer, Gastric Cancer, Gastrointestinal Cancers, Gastrointestinal-Carcinoid-Tumour, Genitourinary Cancers, Germ Cell Tumours, Gestational-Trophoblastic-Disease, Glioma, Gynaecological Cancers, Haematological Malignancies, Hairy Cell Leukaemia, Head and Neck Cancer, Hepatocellular Cancer, Hereditary Breast Cancer, Histiocytosis, Hodgkin's Disease, Human Papillomavirus, Hydatidiform mole, Hypercalcemia, Hypopharynx Cancer, IntraOcular Melanoma, Islet cell cancer, Kaposi's sarcoma, Kidney Cancer, Langerhan's-Cell-Histiocytosis, Laryngeal Cancer, Leiomyosarcoma, Leukaemia,

Li-Fraumeni Syndrome, Lip Cancer, Liposarcoma, Liver Cancer, Lung Cancer, Lymphedema, Lymphoma, Hodgkin's Lymphoma, Non-Hodgkin's Lymphoma, Male Breast Cancer, Malignant-Rhabdoid-Tumour-of-Kidney, Medulloblastoma, Melanoma, Merkel Cell Cancer, Mesothelioma, Metastatic Cancer, Mouth Cancer, Multiple Endocrine Neoplasia, Mycosis Fungoides, Myelodysplastic Syndromes, Myeloma, Myeloproliferative Disorders, Nasal Cancer, Nasopharyngeal Cancer, Nephroblastoma, Neuroblastoma, Neurofibromatosis, Nijmegen Breakage Syndrome, Non-Melanoma Skin Cancer, Non-Small-Cell-Lung-Cancer-(NSCLC), Ocular Cancers, Oesophageal Cancer, Oral cavity Cancer, Oropharynx Cancer, Osteosarcoma, Ostomy Ovarian Cancer, Pancreas Cancer, Paranasal Cancer, Parathyroid Cancer, Parotid Gland Cancer, Penile Cancer, Peripheral-Neuroectodermal-Tumours, Pituitary Cancer, Polycythemia vera, Prostate Cancer, Rare-cancers-and-associated-disorders, Renal Cell Carcinoma, Retinoblastoma, Rhabdomyosarcoma, Rothmund-Thomson Syndrome, Salivary Gland Cancer, Sarcoma, Schwannoma, Sezary syndrome, Skin Cancer, Small Cell Lung Cancer (SCLC), Small Intestine Cancer, Soft Tissue Sarcoma, Spinal Cord Tumours, Squamous-Cell-Carcinoma-(skin), Stomach Cancer, Synovial sarcoma, Testicular Cancer, Thymus Cancer, Thyroid Cancer, Transitional-Cell-Cancer-(bladder), Transitional-Cell-Cancer-(renal-pelvis-/-ureter), Trophoblastic Cancer, Urethral Cancer, Urinary System Cancer, Uroplakins, Uterine sarcoma, Uterus Cancer, Vaginal Cancer, Vulva Cancer, Waldenstrom's-Macroglobulinemia, Wilms' Tumour. In certain embodiments, the cancer or tumour relates to melanoma. Illustrative examples of melanoma-related antigens include melanocyte differentiation antigen (e.g., gp100, MART, TRP-1, Tyros, TRP2, MC1R, MUC1F, MUC1R or a combination thereof) and melanoma-specific antigens (e.g., BAGE, GAGE-1, gp100In4, MAGE-1 (e.g., GenBank Accession No. X54156 and AA494311), MAGE-3, MAGE4, PRAME, TRP2IN2, NYNSO1a, NYNSO1b, LAGE1, p97 melanoma antigen (e.g., GenBank Accession No. M12154) or a combination thereof). Other tumour-specific antigens include the Ras peptide and p53 peptide associated with advanced cancers, MUC1-KLH antigen associated with breast carcinoma (e.g., GenBank Accession No. J03651), CEA (carcinoembryonic antigen) associated with colorectal cancer (e.g., GenBank Accession No. X98311), gp100 (e.g., GenBank Accession No. S73003) and the PSA antigen with prostate cancer (e.g., GenBank Accession No. X14810). The p53 gene sequence is known (See e.g., Harris *et al.*, 1986 *Mol. Cell. Biol.* 6:4650-4656) and is deposited with GenBank under Accession No. M14694.

[0070] Foreign antigens are suitably selected from transplantation antigens, allergens as well as antigens from pathogenic organisms. Transplantation antigens can be derived from donor cells or tissues from e.g., heart, lung, liver, pancreas, kidney, neural graft components, or from the donor antigen-presenting cells bearing MHC loaded with self antigen in the absence of exogenous antigen.

[0071] Non-limiting examples of allergens include Fel d 1 (i.e., the feline skin and salivary gland allergen of the domestic cat *Felis domesticus*, the amino acid sequence of which is disclosed International Publication WO 91/06571), Der p I, Der p II, Der fI or Der fII (i.e., the major protein allergens from the house dust mite dermatophagoides, the amino acid sequence of which is disclosed in International Publication WO 94/24281). Other allergens may be derived, for example from the following: grass, tree and weed (including ragweed) pollens; fungi and moulds; foods such as fish, shellfish, crab, lobster, peanuts, nuts, wheat gluten, eggs and milk; stinging insects such as bee, wasp, and hornet and the chirnomidae (non-biting midges); other insects such as the housefly, fruitfly, sheep blow fly, screw worm fly, grain weevil, silkworm, honeybee, non-biting midge larvae, bee moth larvae, mealworm, cockroach and larvae of *Tenibrio molitor* beetle; spiders and mites, including the house dust mite; allergens found in the dander, urine, saliva, blood or other bodily fluid of mammals such as cat, dog, cow, pig, sheep, horse, rabbit, rat, guinea pig, mouse and gerbil; airborne particulates in general; latex; and protein detergent additives.

[0072] Exemplary pathogenic organisms include, but are not limited to, viruses, bacteria, fungi parasites, algae and protozoa and amoebeae. Illustrative examples of viruses include viruses responsible for diseases including, but not limited to, measles, mumps, rubella, poliomyelitis, hepatitis A, B (e.g., GenBank Accession No. E02707), and C (e.g., GenBank Accession No. E06890), as well as other hepatitis viruses, influenza, adenovirus (e.g., types 4 and 7), rabies (e.g., GenBank Accession No. M34678), yellow fever, Epstein-Barr virus and other herpesviruses such as papillomavirus, Ebola virus, influenza virus, Japanese encephalitis (e.g., GenBank Accession No. E07883), dengue (e.g., GenBank Accession No. M24444), hantavirus, sendai virus, respiratory syncytial virus, othromyxoviruses, vesicular stomatitis virus, visna virus, cytomegalovirus and human immunodeficiency virus (HIV) (e.g., GenBank Accession No. U18552). Any suitable antigen derived from such viruses are useful in the practice of the present invention. For example, illustrative retroviral antigens derived from HIV include, but are not limited to, antigens such as gene products of the *gag*, *pol*, and *env* genes, the Nef

protein, reverse transcriptase, and other HIV components. Illustrative examples of hepatitis viral antigens include, but are not limited to, antigens such as the S, M, and L proteins of hepatitis B virus, the pre-S antigen of hepatitis B virus, and other hepatitis, e.g., hepatitis A, B, and C, viral components such as hepatitis C viral RNA. Illustrative examples of influenza viral antigens include; but are not limited to, antigens such as hemagglutinin and neuraminidase and other influenza viral components. Illustrative examples of measles viral antigens include, but are not limited to, antigens such as the measles virus fusion protein and other measles virus components. Illustrative examples of rubella viral antigens include, but are not limited to, antigens such as proteins El and E2 and other rubella virus components; rotaviral antigens such as VP7sc and other rotaviral components. Illustrative examples of cytomegaloviral antigens include, but are not limited to, antigens such as envelope glycoprotein B and other cytomegaloviral antigen components. Non-limiting examples of respiratory syncytial viral antigens include antigens such as the RSV fusion protein, the M2 protein and other respiratory syncytial viral antigen components. Illustrative examples of herpes simplex viral antigens include, but are not limited to, antigens such as immediate early proteins, glycoprotein D, and other herpes simplex viral antigen components. Non-limiting examples of varicella zoster viral antigens include antigens such as 9PI, gpII, and other varicella zoster viral antigen components. Non-limiting examples of Japanese encephalitis viral antigens include antigens such as proteins E, M-E, M-E-NS 1, NS 1, NS 1-NS2A, 80%E, and other Japanese encephalitis viral antigen components. Illustrative examples of rabies viral antigens include, but are not limited to, antigens such as rabies glycoprotein, rabies nucleoprotein and other rabies viral antigen components. Illustrative examples of papillomavirus antigens include, but are not limited to, the L1 and L2 capsid proteins as well as the E6/E7 antigens associated with cervical cancers, *See* Fundamental Virology, Second Edition, eds. Fields, B.N. and Knipe, D.M., 1991, Raven Press, New York, for additional examples of viral antigens.

[0073] Illustrative examples of fungi include *Acremonium* spp., *Aspergillus* spp., *Basidiobolus* spp., *Bipolaris* spp., *Blastomyces dermatidis, Candida* spp., *Cladophialophora carrionii, Coccoidiodes immitis, Conidiobolus* spp., *Cryptococcus* spp., *Curvularia* spp., *Epidermophyton* spp., *Exophiala jeanselmei, Exserohilum* spp., *Fonsecaea compacta, Fonsecaea pedrosoi, Fusarium oxysporum, Fusarium solani, Geotrichum candidum, Histoplasma capsulatum* var. *capsulatum, Histoplasma capsulatum* var. *duboisii, Hortaea werneckii, Lacazia loboi, Lasiodiplodia theobromae, Leptosphaeria*

senegalensis, Madurella grisea, Madurella mycetomatis, Malassezia furfur, Microsporum spp., *Neotestudina rosatii, Onychocola canadensis, Paracoccidioides brasiliensis, Phialophora verrucosa, Piedraia hortae, Piedra iahortae, Pityriasis versicolor, Pseudallesheria boydii, Pyrenochaeta romeroi, Rhizopus arrhizus, Scopulariopsis brevicaulis, Scytalidium dimidiatum, Sporothrix schenckii, Trichophyton* spp., *Trichosporon* spp., Zygomcete fungi, *Absidia corymbifera, Rhizomucor pusillus* and *Rhizopus arrhizus*. Thus, illustrative fungal antigens that can be used in the compositions and methods of the present invention include, but are not limited to, candida fungal antigen components; histoplasma fungal antigens such as heat shock protein 60 (HSP60) and other histoplasma fungal antigen components; cryptococcal fungal antigens such as capsular polysaccharides and other cryptococcal fungal antigen components; coccidiodes fungal antigens such as spherule antigens and other coccidiodes fungal antigen components; and tinea fungal antigens such as trichophytin and other coccidiodes fungal antigen components.

[0074] Illustrative examples of bacteria include bacteria that are responsible for diseases including, but not restricted to, diphtheria (e.g., *Corynebacterium diphtheria*), pertussis (e.g., *Bordetella pertussis*, GenBank Accession No. M35274), tetanus (e.g., *Clostridium tetani*, GenBank Accession No. M64353), tuberculosis (e.g., *Mycobacterium tuberculosis*), bacterial pneumonias (e.g., *Haemophilus influenzae*.), cholera (e.g., *Vibrio cholerae*), anthrax (e.g., *Bacillus anthracis*), typhoid, plague, shigellosis (e.g., *Shigella dysenteriae*), botulism (e.g., *Clostridium botulinum*), salmonellosis (e.g., GenBank Accession No. L03833), peptic ulcers (e.g., *Helicobacter pylori*), Legionnaire's Disease, Lyme disease (e.g., GenBank Accession No. U59487), Other pathogenic bacteria include *Escherichia coli, Clostridium perfringens, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes*. Thus, bacterial antigens which can be used in the compositions and methods of the invention include, but are not limited to: pertussis bacterial antigens such as pertussis toxin, filamentous hemagglutinin, pertactin, F M2, FIM3, adenylate cyclase and other pertussis bacterial antigen components; diphtheria bacterial antigens such as diphtheria toxin or toxoid and other diphtheria bacterial antigen components; tetanus bacterial antigens such as tetanus toxin or toxoid and other tetanus bacterial antigen components, streptococcal bacterial antigens such as M proteins and other streptococcal bacterial antigen components; gram-negative bacilli bacterial antigens such as lipopolysaccharides and other gram-negative bacterial antigen components; *Mycobacterium tuberculosis* bacterial antigens such as mycolic acid, heat

shock protein 65 (HSP65), the 30kDa major secreted protein, antigen 85A and other mycobacterial antigen components; *Helicobacter pylori* bacterial antigen components, pneumococcal bacterial antigens such as pneumolysin, pneumococcal capsular polysaccharides and other pnermiococcal bacterial antigen components; *Haemophilus influenza* bacterial antigens such as capsular polysaccharides and other *Haemophilus influenza* bacterial antigen components; anthrax bacterial antigens such as anthrax protective antigen and other anthrax bacterial antigen components; rickettsiae bacterial antigens such as rompA and other rickettsiae bacterial antigen component. Also included with the bacterial antigens described herein are any other bacterial, mycobacterial, mycoplasmal, rickettsial, or chlamydial antigens.

[0075] Illustrative examples of protozoa include protozoa that are responsible for diseases including, but not limited to, malaria (e.g., GenBank Accession No. X53832), hookworm, onchocerciasis (e.g., GenBank Accession No. M27807), schistosomiasis (e.g., GenBank Accession No. LOS 198), toxoplasmosis, trypanosomiasis, leishmaniasis, giardiasis (GenBank Accession No. M33641), amoebiasis, filariasis (e.g., GenBank Accession No. J03266), borreliosis, and trichinosis. Thus, protozoal antigens which can be used in the compositions and methods of the invention include, but are not limited to: plasmodium falciparum antigens such as merozoite surface antigens, sporozoite surface antigens, circumsporozoite antigens, gametocyte/gamete surface antigens, blood-stage antigen pf 155/RESA and other plasmodial antigen components; toxoplasma antigens such as SAG-1, p30 and other toxoplasmal antigen components; schistosomae antigens such as glutathione-S-transferase, paramyosin, and other schistosomal antigen components; leishmania major and other leishmaniae antigens such as gp63, lipophosphoglycan and its associated protein and other leishmanial antigen components; and trypanosoma cruzi antigens such as the 75-77kDa antigen, the 56kDa antigen and other trypanosomal antigen components.

[0076] The present invention also contemplates toxin components as antigens. Illustrative examples of toxins include, but are not restricted to, staphylococcal enterotoxins, toxic shock syndrome toxin; retroviral antigens (e.g., antigens derived from HIV), streptococcal antigens, staphylococcal enterotoxin-A (SEA), staphylococcal enterotoxin-B (SEB), staphylococcal enterotoxin$_{1-3}$ (SE_{1-3}), staphylococcal enterotoxin-D (SED), staphylococcal enterotoxin-E (SEE) as well as toxins derived from mycoplasma, mycobacterium, and herpes viruses.

[0077] The antigen corresponding to at least a portion of the target antigen may be isolated from a natural source or may be prepared by recombinant techniques as known in the art. For example, peptide antigens can be eluted from the MHC and other presenting molecules of antigen-presenting cells obtained from a cell population or tissue for which a modified immune response is desired. The eluted peptides can be purified using standard protein purification techniques known in the art (Rawson *et al.*, 2000, Cancer Res *60*(16), 4493-4498. If desired, the purified peptides can be sequenced and synthetic versions of the peptides produced using standard protein synthesis techniques as for example described below. Alternatively, crude antigen preparations can be produced by isolating a sample of a cell population or tissue for which a modified immune response is desired, and either lysing the sample or subjecting the sample to conditions that will lead to the formation of apoptotic cells (e.g., irradiation with ultra violet or with gamma rays, viral infection, cytokines or by depriving cells of nutrients in the cell culture medium, incubation with hydrogen peroxide, or with drugs such as dexamethasone, ceramide chemotherapeutics and anti-hormonal agents such as Lupron or Tamoxifen). The lysate or the apoptotic cells can then be used as a source of crude antigen for use in soluble form or for contact with antigen-presenting cells as described in more detail below.

[0078] When the antigen is known, it may be conveniently prepared in recombinant form using standard protocols as for example described in: Sambrook, *et al.*, MOLECULAR CLONING. A LABORATORY MANUAL (Cold Spring Harbor Press, 1989), in particular Sections 16 and 17; Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (John Wiley & Sons, Inc. 1994-1998), in particular Chapters 10 and 16; and Coligan *et al.*, CURRENT PROTOCOLS IN PROTEIN SCIENCE (John Wiley & Sons, Inc. 1995-1997), in particular Chapters 1, 5 and 6. Typically, an antigen may be prepared by a procedure including the steps of (a) providing an expression vector from which the target antigen or analogue or mimetic thereof is expressible; (b) introducing the vector into a suitable host cell; (c) culturing the host cell to express recombinant polypeptide from the vector; and (d) isolating the recombinant polypeptide.

[0079] In general, the expression vector will comprise an antigen-encoding polynucleotide which is operably connected to a regulatory polynucleotide. The antigen-encoding polynucleotide can be constructed from any suitable parent polynucleotide that codes for an antigen that corresponds to the target antigen of interest. The parent polynucleotide is suitably a natural gene or portion thereof. However, it is possible that the

parent polynucleotide is not naturally-occurring but has been engineered using recombinant techniques. The regulatory polynucleotide suitably comprises transcriptional and/or translational control sequences, which will generally be appropriate for the host cell used for expression of the antigen-encoding polynucleotide. Typically, the transcriptional and translational regulatory control sequences include, but are not limited to, a promoter sequence, a 5' non-coding region, a *cis*-regulatory region such as a functional binding site for transcriptional regulatory protein or translational regulatory protein, an upstream open reading frame, transcriptional start site, translational start site, and/or nucleotide sequence which encodes a leader sequence, termination codon, translational stop site and a 3' non-translated region. Constitutive or inducible promoters as known in the art are contemplated by the invention. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter. Promoter sequences contemplated by the present invention may be native to the host cell to be introduced or may be derived from an alternative source, where the region is functional in the host cell.

[0080] The expression vector may also comprise a 3' non-translated sequence, which usually refers to that portion of a gene comprising a DNA segment that contains a polyadenylation signal and any other regulatory signals capable of effecting mRNA processing or gene expression. The polyadenylation signal is characterised by effecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. Polyadenylation signals are commonly recognised by the presence of homology to the canonical form 5' AATAAA-3' although variations are not uncommon. The 3' non-translated regulatory DNA sequence typically includes from about 50 to 1,000 nucleotide base pairs and may contain transcriptional and translational termination sequences in addition to a polyadenylation signal and any other regulatory signals capable of effecting mRNA processing or gene expression.

[0081] In certain embodiments, the expression vector further contains a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

[0082] The expression vector may also include a fusion partner (typically provided by the expression vector) so that the recombinant polypeptide is expressed as a fusion polypeptide with the fusion partner. The main advantage of fusion partners is that they assist identification and/or purification of said fusion polypeptide. Well known examples of fusion partners include, but are not limited to, glutathione-S-transferase (GST), Fc

portion of human IgG, maltose binding protein (MBP) and hexahistidine (HIS_6), which are particularly useful for isolation of the fusion polypeptide by affinity chromatography. For the purposes of fusion polypeptide purification by affinity chromatography, relevant matrices for affinity chromatography are glutathione-, amylose-, and nickel- or cobalt-conjugated resins respectively. Many such matrices are available in "kit" form, such as the QIAexpress™ system (Qiagen) useful with (HIS_6) fusion partners and the Pharmacia GST purification system. Preferably, the fusion partners also have protease cleavage sites, such as for Factor X_a or Thrombin, which allow the relevant protease to partially digest the fusion polypeptide of the invention and thereby liberate the recombinant polypeptide of the invention therefrom. The liberated polypeptide can then be isolated from the fusion partner by subsequent chromatographic separation. Fusion partners also include within their scope "epitope tags", which are usually short peptide sequences for which a specific antibody is available. Well known examples of epitope tags for which specific monoclonal antibodies are readily available include c-Myc, influenza virus haemagglutinin and FLAG tags.

[0083] The step of introducing the expression vector into the host cell may be effected by any suitable method including transfection, transduction of viral vectors, including adenoviral, modified lentiviral and other retroviral vectors, and transformation, the choice of which will be dependent on the host cell employed. Such methods are well known to those of skill in the art.

[0084] Recombinant polypeptides may be produced by culturing a host cell transformed with the expression vector under conditions appropriate for protein expression, which will vary with the choice of expression vector and the host cell. This is easily ascertained by one skilled in the art through routine experimentation. Suitable host cells for expression may be prokaryotic or eukaryotic. One preferred host cell for expression of a polypeptide according to the invention is a bacterium. The bacterium used may be *Escherichia coli*. Alternatively, the host cell may be an insect cell such as, for example, *SF9* cells that may be utilised with a baculovirus expression system.

[0085] Alternatively, the antigen can be synthesised using solution synthesis or solid phase synthesis as described, for example, by Atherton and Sheppard (Solid Phase Peptide Synthesis: A Practical Approach, IRL Press at Oxford University Press, Oxford, England, 1989) or by Roberge *et al.* (1995, *Science* **269**: 202). The amino acids of the synthesised antigens can be non-naturally occurring or naturally occurring amino acid. Examples of unnatural amino acids and derivatives during peptide synthesis include but are not limited

to, use of 4-amino butyric acid, 6-aminohexanoic acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 4-amino-3-hydroxy-6-methylheptanoic acid, t-butylglycine, norleucine, norvaline, phenylglycine, ornithine, sarcosine, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acids contemplated by the present invention is shown in TABLE B.

TABLE B

Non-conventional amino acid	Non-conventional amino acid
α-aminobutyric acid	L-N-methylalanine
α-amino-α-methylbutyrate	L-N-methylarginine
aminocyclopropane-carboxylate	L-N-methylasparagine
aminoisobutyric acid	L-N-methylaspartic acid
aminonorbornyl-carboxylate	L-N-methylcysteine
cyclohexylalanine	L-N-methylglutamine
cyclopentylalanine	L-N-methylglutamic acid
L-N-methylisoleucine	L-N-methylhistidine
D-alanine	L-N-methylleucine
D-arginine	L-N-methyllysine
D-aspartic acid	L-N-methylmethionine
D-cysteine	L-N-methylnorleucine
D-glutamate	L-N-methylnorvaline
D-glutamic acid	L-N-methylornithine
D-histidine	L-N-methylphenylalanine
D-isoleucine	L-N-methylproline
D-leucine	L-N-medlylserine
D-lysine	L-N-methylthreonine
D-methionine	L-N-methyltryptophan
D-ornithine	L-N-methyltyrosine

Non-conventional amino acid	*Non-conventional amino acid*
D-phenylalanine	L-N-methylvaline
D-proline	L-N-methylethylglycine
D-serine	L-N-methyl-t-butylglycine
D-threonine	L-norleucine
D-tryptophan	L-norvaline
D-tyrosine	α-methyl-aminoisobutyrate
D-valine	α-methyl-γ-aminobutyrate
D-α-methylalanine	α-methylcyclohexylalanine
D-α-methylarginine	α-methylcylcopentylalanine
D-α-methylasparagine	α-methyl-α-napthylalanine
D-α-methylaspartate	α-methylpenicillamine
D-α-methylcysteine	N-(4-aminobutyl)glycine
D-α-methylglutamine	N-(2-aminoethyl)glycine
D-α-methylhistidine	N-(3-aminopropyl)glycine
D-α-methylisoleucine	N-amino-α-methylbutyrate
D-α-methylleucine	α-napthylalanine
D-α-methyllysine	N-benzylglycine
D-α-methylmethionine	N-(2-carbamylediyl)glycine
D-α-methylornithiine	N-(carbamylmethyl)glycine
D-α-methylphenylalanine	N-(2-carboxyethyl)glycine
D-α-methylproline	N-(carboxymethyl)glycine
D-α-methylserine	N-cyclobutylglycine
D-α-methylthreonine	N-cycloheptylglycine
D-α-methyltryptophan	N-cyclohexylglycine
D-α-methyltyrosine	N-cyclodecylglycine
L-α-methylleucine	L-α-methyllysine

Non-conventional amino acid	*Non-conventional amino acid*
L-α-methylmethionine	L-α-methylnorleucine
L-α-methylnorvatine	L-α-methylornithine
L-α-methylphenylalanine	L-α-methylproline
L-α-methylserine	L-α-methylthreonine
L-α-methyltryptophan	L-α-methyltyrosine
L-α-methylvaline	L-N-methylhomophenylalanine
N-(N-(2,2-diphenylethyl carbamylmethyl)glycine	N-(N-(3,3-diphenylpropyl carbamylmethyl)glycine
1-carboxy-1-(2,2-diphenyl-ethyl amino)cyclopropane	

[0086] The invention also contemplates modifying peptide antigens using ordinary molecular biological techniques so as to alter their resistance to proteolytic degradation or to optimise solubility properties or to render them more suitable as an immunogenic agent.

[0087] Peptide antigens may be of any suitable size that can be utilised to stimulate or inhibit an immune response to a target antigen of interest. A number of factors can influence the choice of peptide size. For example, the size of a peptide can be chosen such that it includes, or corresponds to the size of, T cell epitopes and/or B cell epitopes, and their processing requirements. Practitioners in the art will recognise that class I-restricted T cell epitopes are typically between 8 and 10 amino acid residues in length and if placed next to unnatural flanking residues, such epitopes can generally require 2 to 3 natural flanking amino acid residues to ensure that they are efficiently processed and presented. Class II-restricted T cell epitopes usually range between 12 and 25 amino acid residues in length and may not require natural flanking residues for efficient proteolytic processing although it is believed that natural flanking residues may play a role. Another important feature of class II-restricted epitopes is that they generally contain a core of 9-10 amino acid residues in the middle which bind specifically to class II MHC molecules with flanking sequences either side of this core stabilising binding by associating with conserved structures on either side of class II MHC antigens in a sequence independent manner. Thus the functional region of class II-restricted epitopes is typically less than about 15 amino acid residues long. The size of linear B cell epitopes and the factors

effecting their processing, like class II-restricted epitopes, are quite variable although such epitopes are frequently smaller in size than 15 amino acid residues. From the foregoing, it is advantageous, but not essential, that the size of the peptide is at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30 amino acid residues. Suitably, the size of the peptide is no more than about 500, 200, 100, 80, 60, 50, 40 amino acid residues. In certain advantageous embodiments, the size of the peptide is sufficient for presentation by an antigen-presenting cell of a T cell and/or a B cell epitope contained within the peptide.

[0088] Criteria for identifying and selecting effective antigenic peptides (e.g., minimal peptide sequences capable of eliciting an immune response) can be found in the art. For example, Apostolopoulos *et al.* (2000, *Curr. Opin. Mol. Ther.* 2:29-36) discusses the strategy for identifying minimal antigenic peptide sequences based on an understanding of the three dimensional structure of an antigen-presenting molecule and its interaction with both an antigenic peptide and T-cell receptor. Shastri (1996, *Curr. Opin. Immunol.* 8:271-277) discloses how to distinguish rare peptides that serve to activate T cells from the thousands peptides normally bound to MHC molecules.

[0089] The antigen corresponding to at least a portion of the target antigen may be in soluble form or it may be presented by an antigen-presenting cell as described in more detail below.

2.3 Antigen-presenting cell embodiments

[0090] The present invention also contemplates the use of antigen-presenting cells, which present an antigen corresponding to at least a portion of the target antigen, in the compositions of the present invention. Such antigen-presenting cells include professional or facultative antigen-presenting cells. Professional antigen-presenting cells function physiologically to present antigen in a form that is recognised by specific T cell receptors so as to stimulate or anergise a T lymphocyte or B lymphocyte mediated immune response. Professional antigen-presenting cells not only process and present antigens in the context of the major histocompatibility complex (MHC), but also possess the additional immunoregulatory molecules required to complete T cell activation or induce a tolerogenic response. Professional antigen-presenting cells include, but are not limited to, macrophages, monocytes, B lymphocytes, cells of myeloid lineage, including monocytic-granulocytic-DC precursors, marginal zone Kupffer cells, microglia, T cells, Langerhans cells and dendritic cells including interdigitating dendritic cells and follicular dendritic cells. Non-professional or facultative antigen-presenting cells typically lack one or more of

the immunoregulatory molecules required to complete T lymphocyte activation or anergy. Examples of non-professional or facultative antigen-presenting cells include, but are not limited to, activated T lymphocytes, eosinophils, keratinocytes, astrocytes, follicular cells, microglial cells, thymic cortical cells, endothelial cells, Schwann cells, retinal pigment epithelial cells, myoblasts, vascular smooth muscle cells, chondrocytes, enterocytes, thymocytes, kidney tubule cells and fibroblasts. In some embodiments, the antigen-presenting cell is selected from monocytes, macrophages, B lymphocytes, cells of myeloid lineage, dendritic cells or Langerhans cells. In certain advantageous embodiments, the antigen-presenting cell expresses CD11c and includes a dendritic cell. In some embodiments the antigen-presenting cell stimulates an immune response. In other embodiments, the antigen-presenting cell induces a tolerogenic response. Antigen-presenting cells for stimulating an immune response to an antigen or group of antigens, or for inducing a tolerogenic response including the induction of an anergic response or the suppression of a future or existing immune response, to an antigen or group of antigens may be prepared according to any suitable method known to the skilled practitioner. Illustrative methods for preparing antigen-presenting cells for stimulating antigen-specific immune responses are described by Albert *et al.* (International Publication WO 99/42564), Takamizawa *et al.* (1997, *J Immunol*, **158**(5): 2134-2142), Thomas and Lipsky (1994, *J Immunol*, **153**(9):4016-4028), O'Doherty *et al.* (1994, *Immunology*, **82**(3):487-93), Fearnley *et al.* (1997, *Blood*, **89**(10): 3708-3716), Weissman *et al.* (1995, *Proc Natl Acad Sci U S A*, **92**(3):826-830), Freudenthal and Steinman (1990, *Proc Natl Acad Sci U S A*, **87**(19):7698-7702), Romani *et al.* (1996, *J Immunol Methods*, **196**(2): 137-151), Reddy *et al.* (1997, *Blood*, **90**(9):3640-3646), Thumher *et al.* (1997, *Exp Hematol*, **25**(3):232-237), Caux *et al.* (1996, *J Exp Med*, **184**(2):695-706; 1996, *Blood*, **87**(6):2376-85), Luft *et al.* (1998, *Exp Hematol*, **26**(6):489-500; 1998, *J Immunol*, **161**(4):1947-1953), Cella *et al.* (1999, *J Exp Med*, **189**(5): 821-829;1997, *Nature*, **388**(644):782-787; 1996, *J Exp Med*, **184**(2):747-572), Ahonen *et al.* (1999, *Cell Immunol*, **197**(1):62-72) and Piemonti *et al.* (1999, *J Immunol*, **162**(11):6473-6481). Illustrative methods for preparing antigen-presenting cells for inhibiting immune responses or for inducing tolerogenic responses are described by Ichim *et al.* (2003, *Transplant Immunol* 11:295-306) including methods for generating 'killer dendritic cells (DC)' by transfections with FasL, generating immature DC by inhibition of NF-κB, generation of MHC-inhibited DC by treatment with CLIP and generation of IL-12 gene-silenced CD by post-transcriptional gene silencing or RNA interference.

[0091] In some embodiments, the antigen-presenting cells are isolated from a host, treated and then re-introduced or reinfused into the host. Conveniently, antigen-presenting cells can be obtained from the host to be treated either by surgical resection, biopsy, blood sampling, or other suitable technique. Such cells are referred to herein as "autologous" cells. In other embodiments, the antigen-presenting cells or cell lines are prepared and/or cultured from a different source than the host. Such cells are referred to herein as "allogeneic" cells. Desirably, allogeneic antigen-presenting cells or cell lines will share major and/or minor histocompatibility antigens to potential recipients (also referred to herein as 'generic' antigen-presenting cells or cell lines). In certain advantageous embodiments of this type, the generic antigen-presenting cells or cell lines comprise major histocompatibility (MHC) class I antigens compatible with a high percentage of the population (i.e., at least 10, 20, 30, 40, 50, 60, 70, 75, 80, 85, 90, 92, 94 or 98%) that is susceptible or predisposed to a particular condition. Suitably, the generic antigen-presenting cells or cell lines naturally express an immunostimulatory molecule as described herein, especially an immunostimulatory membrane molecule, at levels sufficient to trigger an immune response, desirably a T lymphocyte immune response (e.g., a cytotoxic T lymphocyte immune response), in the intended host. In certain embodiments, the antigen-presenting cells or cell lines are highly susceptible to treatment with at least one IFN as described herein (i.e., implied high level expression of class I HLA).

[0092] In some embodiments, antigen-presenting cells are made antigen-specific by a process including contacting or 'pulsing' the antigen-presenting cells with an antigen that corresponds to at least a portion of the target antigen for a time and under conditions sufficient to permit the antigen to be internalised by the antigen-presenting cells; and culturing the antigen-presenting cells so contacted for a time and under conditions sufficient for the antigen to be processed for presentation by the antigen-presenting cells. The pulsed cells can then be used to stimulate autologous or allogeneic T cells *in vitro* or *in vivo*. The amount of antigen to be placed in contact with antigen-presenting cells can be determined empirically by persons of skill in the art. Typically antigen-presenting cells are incubated with antigen for about 1 to 6 hr at 37° C. Usually, for purified antigens and peptides, 0.1-10 μg/mL is suitable for producing antigen-specific antigen-presenting cells. The antigen should be exposed to the antigen-presenting cells for a period of time sufficient for those cells to internalise the antigen. The time and dose of antigen necessary for the cells to internalise and present the processed antigen may be determined using pulse-chase protocols in which exposure to antigen is followed by a washout period and

exposure to a read-out system e.g., antigen reactive T cells. Once the optimal time and dose necessary for cells to express processed antigen on their surface is determined, a protocol may be used to prepare cells and antigen for inducing tolerogenic responses. Those of skill in the art will recognise in this regard that the length of time necessary for an antigen-presenting cell to present an antigen may vary depending on the antigen or form of antigen employed, its dose, and the antigen-presenting cell employed, as well as the conditions under which antigen loading is undertaken. These parameters can be determined by the skilled artisan using routine procedures.

[0093] The delivery of exogenous antigen to an antigen-presenting cell can be enhanced by methods known to practitioners in the art. For example, several different strategies have been developed for delivery of exogenous antigen to the endogenous processing pathway of antigen-presenting cells, especially dendritic cells. These methods include insertion of antigen into pH-sensitive liposomes (Zhou and Huang, 1994, *Immunomethods*, 4:229-235), osmotic lysis of pinosomes after pinocytic uptake of soluble antigen (Moore *et al.*, 1988, *Cell*, 54:777-785), coupling of antigens to potent adjuvants (Aichele *et al.*, 1990, *J. Exp. Med.*, 171: 1815-1820; Gao *et al.*, 1991, *J. Immunol.*, 147: 3268-3273; Schulz *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88: 991-993; Kuzu *et al.*, 1993, *Euro. J. Immunol.*, 23: 1397-1400; and Jondal *et al.*, 1996, *Immunity* 5: 295-302) and apoptotic cell delivery of antigen (Albert *et al.* 1998, *Nature* 392:86-89; Albert *et al.* 1998, *Nature Med.* 4:1321-1324; and in International Publications WO 99/42564 and WO 01/85207). Recombinant bacteria (eg. *E. coli*) or transfected host mammalian cells may be pulsed onto dendritic cells (as particulate antigen, or apoptotic bodies respectively) for antigen delivery. Recombinant chimeric virus-like particles (VLPs) have also been used as vehicles for delivery of exogenous heterologous antigen to the MHC class I processing pathway of a dendritic cell line (Bachmann *et al.*, 1996, *Eur. J. Immunol.*, 26(11): 2595-2600).

[0094] Alternatively, or in addition, an antigen may be linked to, or otherwise associated with, a cytolysin to enhance the transfer of the antigen into the cytosol of an antigen-presenting cell of the invention for delivery to the MHC class I pathway. Exemplary cytolysins include saponin compounds such as saponin-containing Immune Stimulating Complexes (ISCOMs) (see e.g., Cox and Coulter, 1997, *Vaccine* 15(3): 248-256 and U.S. Patent No. 6,352,697), phospholipases (see, e.g., Camilli *et al.*, 1991, *J. Exp. Med.* 173: 751-754), pore-forming toxins (e.g., an alpha-toxin), natural cytolysins of gram-

positive bacteria, such as listeriolysin O (LLO, e.g., Mengaud *et al.*, 1988, *Infect. Immun.* 56: 766-772 and Portnoy *et al.*, 1992, *Infect. Immun.* 60: 2710-2717), streptolysin O (SLO, e.g., Palmer *et al.*, 1998, *Biochemistry* 37(8): 2378-2383) and perfringolysin O (PFO, e.g., Rossjohn *et al.*, *Cell* 89(5): 685-692). Where the antigen-presenting cell is phagosomal, acid activated cytolysins may be advantageously used. For example, listeriolysin exhibits greater pore-forming ability at mildly acidic pH (the pH conditions within the phagosome), thereby facilitating delivery of vacuole (including phagosome and endosome) contents to the cytoplasm (see, e.g., Portnoy *et al.*, *Infect. Immun.* 1992, 60: 2710-2717).

[0095] The cytolysin may be provided together with a pre-selected antigen in the form of a single composition or may be provided as a separate composition, for contacting the antigen-presenting cells. In one embodiment, the cytolysin is fused or otherwise linked to the antigen, wherein the fusion or linkage permits the delivery of the antigen to the cytosol of the target cell. In another embodiment, the cytolysin and antigen are provided in the form of a delivery vehicle such as, but not limited to, a liposome or a microbial delivery vehicle selected from virus, bacterium, or yeast. Suitably, when the delivery vehicle is a microbial delivery vehicle, the delivery vehicle is non-virulent. In a preferred embodiment of this type, the delivery vehicle is a non-virulent bacterium, as for example described by Portnoy *et al.* in U.S. Patent No. 6,287,556, comprising a first polynucleotide encoding a non-secreted functional cytolysin operably linked to a regulatory polynucleotide which expresses the cytolysin in the bacterium, and a second polynucleotide encoding one or more pre-selected antigens. Non-secreted cytolysins may be provided by various mechanisms, e.g., absence of a functional signal sequence, a secretion incompetent microbe, such as microbes having genetic lesions (e.g., a functional signal sequence mutation), or poisoned microbes, etc. A wide variety of nonvirulent, non-pathogenic bacteria may be used; preferred microbes are relatively well characterised strains, particularly laboratory strains of *E. coli*, such as MC4100, MC1061, DH5.alpha., etc. Other bacteria that can be engineered for the invention include well-characterised, nonvirulent, non-pathogenic strains of *Listeria monocytogenes*, *Shigella flexneri*, mycobacterium, *Salmonella*, *Bacillus subtilis*, etc. In a particular embodiment, the bacteria are attenuated to be non-replicative, non-integrative into the host cell genome, and/or non-motile inter- or intra-cellularly.

[0096] The delivery vehicles described above can be used to deliver one or more antigens to virtually any antigen-presenting cell capable of endocytosis of the subject

vehicle, including phagocytic and non-phagocytic antigen-presenting cells. In embodiments when the delivery vehicle is a microbe, the subject methods generally require microbial uptake by the target cell and subsequent lysis within the antigen-presenting cell vacuole (including phagosomes and endosomes).

[0097] In other embodiments, the antigen is produced inside the antigen-presenting cell by introduction of a suitable expression vector as for example described above. The antigen-encoding portion of the expression vector may comprise a naturally-occurring sequence or a variant thereof, which has been engineered using recombinant techniques. In one example of a variant, the codon composition of an antigen-encoding polynucleotide is modified to permit enhanced expression of the antigen in a target cell or tissue of choice using methods as set forth in detail in International Publications WO 99/02694 and WO 00/42215. Briefly, these methods are based on the observation that translational efficiencies of different codons vary between different cells or tissues and that these differences can be exploited, together with codon composition of a gene, to regulate expression of a protein in a particular cell or tissue type. Thus, for the construction of codon-optimised polynucleotides, at least one existing codon of a parent polynucleotide is replaced with a synonymous codon that has a higher translational efficiency in a target cell or tissue than the existing codon it replaces. Although it is preferable to replace all the existing codons of a parent nucleic acid molecule with synonymous codons which have that higher translational efficiency, this is not necessary because increased expression can be accomplished even with partial replacement. Suitably, the replacement step affects 5, 10, 15, 20, 25, 30%, more preferably 35, 40, 50, 60, 70% or more of the existing codons of a parent polynucleotide.

[0098] The expression vector for introduction into the antigen-presenting cell will be compatible therewith such that the antigen-encoding polynucleotide is expressible by the cell. For example, expression vectors of this type can be derived from viral DNA sequences including, but not limited to, adenovirus, adeno-associated viruses, herpes-simplex viruses and retroviruses such as B, C, and D retroviruses as well as spumaviruses and modified lentiviruses. Suitable expression vectors for transfection of animal cells are described, for example, by Wu and Ataai (2000, *Curr. Opin. Biotechnol.* 11(2):205-208), Vigna and Naldini (2000, *J. Gene Med.* 2(5):308-316), Kay, *et al.* (2001, *Nat. Med.* 7(1):33-40), Athanasopoulos, *et al.* (2000, *Int. J. Mol. Med.* 6(4):363-375) and Walther and Stein (2000, *Drugs* 60(2):249-271). The expression vector is introduced into the antigen-

presenting cell by any suitable means which will be dependent on the particular choice of expression vector and antigen-presenting cell employed. Such means of introduction are well-known to those skilled in the art. For example, introduction can be effected by use of contacting (e.g., in the case of viral vectors), electroporation, transformation, transduction, conjugation or triparental mating, transfection, infection membrane fusion with cationic lipids, high-velocity bombardment with DNA-coated microprojectiles, incubation with calcium phosphate-DNA precipitate, direct microinjection into single cells, and the like. Other methods also are available and are known to those skilled in the art. Alternatively, the vectors are introduced by means of cationic lipids, e.g., liposomes. Such liposomes are commercially available (e.g., Lipofectin®, Lipofectamine™, and the like, supplied by Life Technologies, Gibco BRL, Gaithersburg, Md.). It will be understood by persons of skill in the art that the techniques for assembling and expressing antigen-encoding nucleic acid molecules, immunoregulatory molecules and/or cytokines as described herein e.g., synthesis of oligonucleotides, nucleic acid amplification techniques, transforming cells, constructing vectors, expressions system and the like and transducing or otherwise introducing nucleic acid molecules into cells are well established in the art, and most practitioners are familiar with the standard resource materials for specific conditions and procedures.

[0099] In some embodiments, the antigen-specific antigen-presenting cells are obtained by isolating antigen-presenting cells or their precursors from a cell population or tissue to which modification of an immune response is desired. Typically, some of the isolated antigen-presenting cells or precursors will constitutively present antigens or have taken up such antigen *in vivo* that are targets or potential targets of an immune response for which stimulation or inhibition of an immune response is desired. In this instance, the delivery of exogenous antigen is not essential. Alternatively, cells may be derived from biopsies of healthy or diseased tissues, lysed or rendered apoptotic and the pulsed onto antigen-presenting cells (e.g., dendritic cells). In certain embodiments of this type, the antigen-presenting cells are cancer or tumour cells to which an antigen-specific immune response is required. Illustrative examples of cancers or tumour cells include cells of sarcomas and carcinomas, e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal

cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocyfc) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease. In certain embodiments, the cancer or tumour cells are selected from the group melanoma cells and mammary carcinoma cells.

[0100] In some of the above embodiments, the cancer or tumour cells will constitute facultative or non-professional antigen-presenting cells, and may in some instances require further modification to enhance their antigen-presenting functions. In these instances, the antigen-presenting cells are further modified to express one or more immunoregulatory molecules, which include any molecules occurring naturally in animals that may regulate or directly influence immune responses including: proteins involved in antigen processing and presentation such as TAP1/TAP2 transporter proteins, proteosome molecules such as LMP2 and LMP7, heat shock proteins such as gp96, HSP70 and HSP90, and major histocompatibility complex (MHC) or human leucocyte antigen (HLA) molecules; factors that provide co-stimulation signals for T cell activation such as B7 and CD40; factors that provide co-inhibitory signals for direct killing of T cells or induction of T lymphocyte or B lymphocyte anergy or stimulation of T regulatory cell (Treg) generation such as OX-2, programmed death-1 ligand (PD-1L); accessory molecules such as CD83; chemokines; lymphokines and cytokines such as interferons α, β and γ, interleukins (e.g., IL-2, IL-7, IL-12, IL-15, IL-22, etc.), factors stimulating cell growth (e.g., GM-SCF) and other factors (e.g., tumour necrosis factors (TNFs), DC-SIGN, MIP1α, MIP1β and transforming growth factor-β (TGF-β). In certain advantageous embodiments, the immunoregulatory molecules are selected from a B7 molecule (e.g., B7-1, B7-2 or B7-3) and an ICAM molecule (e.g., ICAM-1 and ICAM-2).

[0101] Instead of recombinantly expressing immunoregulatory molecules, antigen-presenting cells expressing the desired immunostimulatory molecule(s) may be isolated or selected from a heterogeneous population of cells. Any method of isolation/selection is contemplated by the present invention, examples of which are known to those of skill in the art. For instance, one can take advantage of one or more particular characteristics of a cell to specifically isolate that cell from a heterogeneous population. Such characteristics include, but are not limited to, anatomical location of a cell, cell density, cell size, cell morphology, cellular metabolic activity, cell uptake of ions such as Ca^{2+}, K^{+}, and H^{+} ions, cell uptake of compounds such as stains, markers expressed on the cell surface, protein fluorescence, and membrane potential. Suitable methods that can be used in this regard include surgical removal of tissue, flow cytometry techniques such as fluorescence-activated cell sorting (FACS), immunoaffinity separation (*e.g.*, magnetic bead separation such as Dynabead™ separation), density separation (*e.g.*, metrizamide, Percoll™, or Ficoll™ gradient centrifugation), and cell-type specific density separation. Desirably, the cells are isolated by flow cytometry or by immunoaffinity separation using an antigen-binding molecule that is immuno-interactive with the immunoregulatory molecule.

[0102] Alternatively, the immunoregulatory molecule can be provided to the antigen-presenting cells in soluble form. In some embodiments of this type, the immunoregulatory molecule is a B7 molecule that lacks a functional transmembrane domain (e.g., that comprises a B7 extracellular domain), non-limiting examples of which are described by McHugh *et al.* (1998, *Clin. Immunol. Immunopathol.* 87(1):50-59), Faas *et al.* (2000, *J. Immunol.* 164(12):6340-6348) and Jeannin *et al.* (2000, *Immunity* 13(3):303-312). In other embodiments of this type, the immunostimulatory protein is a B7 derivative including, but not limited to, a chimeric or fusion protein comprising a B7 molecule, or biologically active fragment thereof, or variant or derivative of these, linked together with an antigen binding molecule such as an immunoglobulin molecule or biologically active fragment thereof. For example, a polynucleotide encoding the amino acid sequence corresponding to the extracellular domain of the B7-1 molecule, containing amino acids from about position 1 to about position 215, is joined to a polynucleotide encoding the amino acid sequences corresponding to the hinge, CH2 and CH3 regions of human Ig Cγ1, using PCR, to form a construct that is expressed as a B7Ig fusion protein. DNA encoding the amino acid sequence corresponding to a B7Ig fusion protein has been deposited with the American Type culture Collection (ATCC) in Rockville, Md., under the Budapest Treaty on May 31, 1991 and accorded accession number 68627. Techniques for making and assembling such

B7 derivatives are disclosed for example by Linsley *et al.* (U.S. Patent No. 5,580,756). Reference also may be made to Sturmhoefel *et al.* (1999, *Cancer Res.* 59: 4964-4972) who disclose fusion proteins comprising the extracellular region of B7-1 or B7-2 fused in frame to the Fc portion of IgG2a.

[0103] The half-life of a soluble immunoregulatory molecule may be prolonged by any suitable procedure if desired. Preferably, such molecules are chemically modified with polyethylene glycol (PEG), including monomethoxy-polyethylene glycol, as for example disclosed by Chapman *et al* (1999, *Nature Biotechnology* 17: 780-783).

[0104] Alternatively, or in addition, the antigen-presenting cells are cultured in the presence of at least one interferon for a time and under conditions sufficient to enhance the antigen presenting function of the cells and washing the cells to remove the interferon(s). In certain advantageous embodiments of this type, the step of culturing may comprise contacting the cells with at least one type I interferon and/or a type II interferon. The at least one type I interferon is suitably selected from the group consisting of an IFN-alpha, an IFN-beta, a biologically active fragment of an IFN-alpha, a biologically active fragment of an IFN-beta, a variant of an IFN-alpha, a variant of an IFN-beta, a variant of a said biologically active fragment, a derivative of an IFN-alpha, a derivative of an IFN-beta, a derivative of a said biologically active fragment, a derivative of a said variant, an analogue of IFN-alpha and an analogue of IFN-beta. Typically, the type II interferon is selected from the group consisting of an IFN-gamma, a biologically active fragment of an IFN-gamma, a variant of an IFN-gamma, a variant of said biologically active fragment, a derivative of an IFN-gamma, a derivative of said biologically active fragment, a derivative of said variant and an analogue of an IFN-gamma. Exemplary methods and conditions for enhancing the antigen-presenting functions of antigen-presenting cells using interferon treatment are described in International Publication No. WO 01/88097.

[0105] In some embodiments, the antigen-presenting cells (e.g., cancer cells) or cell lines are suitably rendered inactive to prevent further proliferation once administered to the subject. Any physical, chemical, or biological means of inactivation may be used, including but not limited to irradiation (generally with at least about 5,000 cGy, usually at least about 10,000 cGy, typically at least about 20,000 cGy); or treatment with mitomycin-C (usually at least 10 μg/mL; more usually at least about 50 μg/mL).

2.4 Immune effector cell embodiments

[0106] The antigen-presenting cells may be obtained or prepared to contain and/or express one or more antigens by any number of means, such that the antigen(s) or processed form(s) thereof, is (are) presented by those cells for potential modulation of other immune cells, including T lymphocytes and B lymphocytes, and particularly for producing T lymphocytes and B lymphocytes that are primed to respond to a specified antigen or group of antigens. In some embodiments, the subject antigen-presenting cells are useful for producing primed T lymphocytes to an antigen or group of antigens. In other embodiments, the subject antigen-specific antigen-presenting cells are useful for producing T lymphocytes that exhibit tolerance/anergy to an antigen or group of antigens. The efficiency of inducing lymphocytes, especially T lymphocytes, to exhibit an immune response or tolerance/anergy to a specified antigen can be determined by any suitable method including, but not limited to, assaying T lymphocyte cytolytic activity *in vitro* using for example antigen-specific antigen-presenting cells as targets of antigen-specific cytolytic T lymphocytes (CTL); assaying antigen-specific T lymphocyte proliferation (see, *e.g.*, Vollenweider and Groseurth, 1992, *J. Immunol. Meth.* **149**: 133-135), measuring B cell response to the antigen using, for example, ELISPOT assays, and ELISA assays; interrogating cytokine profiles; or measuring delayed-type hypersensitivity (DTH) responses by test of skin reactivity to a specified antigen (see, *e.g.*, Chang *et al.* (1993, *Cancer Res.* **53**: 1043-1050). Other methods known to practitioners in the art, which can detect the presence of antigen on the surface of antigen-presenting cells after exposure to the antigen, are also contemplated by the present invention.

[0107] Accordingly, the present invention also provides antigen-specific B or T lymphocytes, especially T lymphocytes, which respond in an antigen-specific fashion to representation of the antigen. In some embodiments, antigen-specific T lymphocytes are produced by contacting an antigen-presenting cell as defined above with a population of T lymphocytes, which may be obtained from any suitable source such as spleen or tonsil/lymph nodes but is preferably obtained from peripheral blood. The T lymphocytes can be used as crude preparations or as partially purified or substantially purified preparations, which are suitably obtained using standard techniques as, for example, described in "Immunochemical Techniques, Part G: Separation and Characterization of Lymphoid Cells" (*Meth. in Enzymol.* **108**, *Edited by* Di Sabato *et al.*, 1984, Academic Press). This includes rosetting with sheep red blood cells, passage across columns of nylon

wool or plastic adherence to deplete adherent cells, immunomagnetic or flow cytometric selection using appropriate monoclonal antibodies is known in the art.

[0108] The preparation of T lymphocytes is contacted with antigen-specific antigen-presenting cells as described herein for an adequate period of time for priming or anergising the T lymphocytes to the antigen or antigens presented by those antigen-presenting cells. This period will usually be at least about 1 day, and up to about 5 days.

[0109] In embodiments employing tolerance or anergy inducing antigen-specific antigen-presenting cells, the antigen-specific anergy induced by such cells desirably involves the induction of one or more types of antigen-specific regulatory lymphocytes, especially regulatory T lymphocytes. Several populations of regulatory T lymphocytes are known to inhibit the response of other (effector) lymphocytes in an antigen-specific manner including, for example, Tr1 lymphocytes, Th3 lymphocytes, Th2 lymphocytes, $CD8^+CD28^-$ regulatory T lymphocytes, $CD4^+CD25^+$ regulatory T lymphocytes, natural killer (NK) T lymphocytes and γδ T lymphocytes.

[0110] Tr1 lymphocytes can emerge after several rounds of stimulation of human blood T cells by allogeneic monocytes in the presence of IL-10. This subpopulation secretes high levels of IL-10 and moderate levels of TGFβ but little IL-4 or IFNγ (Groux *et al.*, 1997, *Nature* **389**:737-742).

[0111] The Th3 regulatory subpopulation refers to a specific subset induced following antigen delivery *via* the oral (or other mucosal) route. They produce predominantly TGFβ, and only low levels of IL-10, IL-4 or IFNγ, and provide specific help for IgA production (Weiner *et al.*, 2001, *Microbes Infect* **3**:947-954). They are able to suppress both Th1 and Th2-type effector T cells.

[0112] Th2 lymphocytes produce high levels of IL-4, IL-5 and IL-10 but low IFNγ and TGFβ. Th2 lymphocytes are generated in response to a relative abundance of IL-4 and lack of IL-12 in the environment at the time of presentation of their cognate peptide ligands (O'Garra and Arai, 2000, *Trends Cell Biol* **10**:542-550). T lymphocyte signalling by CD86 may also be important for generation of Th2 cells (Lenschow *et al.*, 1996, *Immunity* **5**:285-293; Xu *et al.*, 1997, *J Immunol* **159**:4217-4226).

[0113] A distinct $CD8^+CD28^-$ regulatory or "suppressor" subset of T lymphocytes can be induced by repetitive antigenic stimulation *in vitro*. They are MHC class I-restricted, and suppress $CD4^+$ T cell responses.

[0114] $CD4^+CD25^+$ regulatory or "suppressor" subset of T lymphocytes inhibit a variety of autoimmune and inflammatory diseases and they are also efficient in the suppression of alloantigen responses. In particular, these lymphocytes can down-regulate the immune response by affecting T cell responses, antibody production, cytokine secretion and antigen-presenting cells (see for example Suvas *et al.*, 2003, *J Exp Med.* 198(6):889-901; Taams *et al.*, 2003, *Transpl Immunol.* 11(3-4):277-85; Jonuleit *et al.*, 2003, *Transpl Immunol.* 11(3-4):267-76; Green *et al.* 2003, *Proc Natl Acad Sci U S A.* 100(19):10878-10883). CD4+CD25+ regulatory T cells are generated by repetitive antigenic stimulation *in vitro* (Feunou *et al.*, 2003, *J Immunol.* 171(7):3475-84).

[0115] NK T lymphocytes, which express the NK cell marker, CD161, and whose TCR are Vα24JαQ in human and Vα14Jα281 in mouse, are activated specifically by the non-polymorphic CD1d molecule through presentation of a glycolipid antigen (Kawano *et al.*, 1997, *Science* 278:1626-1629). They have been shown to be immunoregulatory in a number of experimental systems. They are reduced in number in several autoimmune models before disease onset, and can reduce incidence of disease upon passive transfer to non-obese diabetic (NOD) mice. Administration of the glycolipid, α-galactosyl ceramide (α-gal cer), presented by CD1d, also results in accumulation of NKT lymphocytes and amelioration of diabetes in these mice (Naumov *et al.*, 2001, *Proc Natl Acad Sci U S A* 98:13838-13843).

[0116] γδ T lymphocytes have been implicated in the downregulation of immune responses in various inflammatory diseases and in the suppression of inflammation associated with induction of mucosal tolerance. The tolerance induced by mucosal antigen was transferable to untreated recipient mice by small numbers of γδ T cells (McMenamin *et al.*, 1995, *J Immunol* 154:4390-4394; McMenamin *et al.*, 1994, *Science* 265:1869-1871). Moreover, mucosal tolerance induction was blocked by the administration of the GL3 antibody that blocks γδ T cell function (Ke *et al.*, 1997, *J Immunol* 158:3610-3618).

[0117] Whether the antigen-specific T lymphocytes are produced in contact with antigen-presenting cells *in vitro* or *in vivo*, the antigen-specific anergy induced by the antigen-presenting cells reflects the inability of the antigen-specific lymphocytes to respond to subsequent restimulation with the specific antigen. These antigen-specific lymphocytes are suitably characterised by production of IL-10 in an antigen-specific manner. IL-10 is a cytokine with potent immunosuppressive properties. IL-10 inhibits antigen-specific T lymphocyte proliferation at different levels. IL-10 inhibits the antigen-

presenting and accessory cell function of professional antigen-presenting cells such as monocytes, dendritic cells and Langerhans cells by downregulation of the expression of MHC class II molecules and of the adhesion and co-stimulatory molecules ICAM-1 and B7.1 and B7.2 (reviewed in Interleukin 10, de Vries and de Waal Malefyt, eds., Landes Co, Austin Tex., 1995). IL-10 also inhibits IL-12 production by these cells. IL-12 promotes T lymphocyte activation and the differentiation of Th1 lymphocytes (D'Andrea, *et al.*, 1993, *J. Exp. Med.* 178:1041-1048; Hsieh *et al.*, 1993, *Science* 260:547-549). In addition, IL-10 directly inhibits T lymphocyte proliferation by inhibiting IL-2 gene transcription and IL-2 production by these cells (reviewed in Interleukin 10, de Vries and de Waal Malefyt, eds., Landes Co, Austin Tex., (1995)), and itself promotes antigen-presenting cells that induce regulatory T cells (U.S. Patent No. 6,277,635). Thus, in some embodiments, the presence of anergic T lymphocytes may be determined by assaying IL-10 production, e.g. by ELISA in cell supernatants, or by flow cytometric analysis of intracellular staining.

2.5 Ancillary components

[0118] In some embodiments the composition further comprises one or more cytokines, which are suitably selected from flt3, SCF, IL-3, IL-6, GM-CSF, G-CSF, TNF-α, IL-4, TNF-β, LT-β, IL-2, IL-7, IL-9, IL-15, IL-13, IL-5, IL-1α, IL-1β, IFN-γ, IL-10, IL-17, IL-16, IL-18, HGF, IL-11, MSP, FasL, TRAIL, TRANCE, LIGHT, TWEAK, CD27L, CD30L, CD40L, APRIL, TALL-1, 4-1BBL, OX40L, GITRL, IGF-I, IGF-II, HGF, MSP, FGF-a, FGF-b, FGF-3-19, NGF, BDNF, NTs, Tpo, Epo, Ang1-4, PDGF-AA, PDGF-BB, VEGF-A, VEGF-B, VEGF-C, VEGF-D, PIGF, EGF, TGF-α, AR, BTC, HRGs, HB-EGF, SMDF, OB, CT-1, CNTF, OSM, SCF, Flt-3L, M-CSF, MK and PTN or their functional, recombinant or chemical equivalents or homologues thereof. Preferably the cytokine is selected from the group consisting of IL-12, IL-3, IL-5, TNF, GMCSF, and IFN-γ.

3. Cell based therapy or prophylaxis

[0119] In accordance with the present invention, the lectin-interactive agent described in Section 3.1 can be administered to a patient, together with the antigen-presenting cells described in Section 3.3 and/or the immune effector cells described in Section 3.4 for modulating an immune response, e.g., for priming an immune response or inducing a tolerogenic immune response to one or more cognate antigens. These cell based compositions are useful, therefore, for treating or preventing a disease or condition that is associated with the presence or aberrant expression of a target antigen. The cells of the

invention can be introduced into a patient by any means (e.g., injection), which produces the desired immune response to an antigen or group of antigens. The cells may be derived from the patient (i.e., autologous cells) or from an individual or individuals who are MHC matched or mismatched (i.e., allogeneic) with the patient. Typically, autologous cells are injected back into the patient from whom the source cells were obtained. The injection site may be subcutaneous, intraperitoneal, intramuscular, intradermal, or intravenous. The cells may be administered to a patient already suffering from a disease or condition or who is predisposed to a disease or condition in sufficient number to treat or prevent or alleviate the symptoms of the disease or condition. The number of cells injected into the patient in need of the treatment or prophylaxis may vary depending on *inter alia*, the antigen or antigens and size of the individual. This number may range for example between about 10^3 and 10^{11}, and usually between about 10^5 and 10^7 cells (e.g., dendritic cells or T lymphocytes). Single or multiple administrations of the cells can be carried out with cell numbers and pattern being selected by the treating physician. The cells should be administered in a pharmaceutically acceptable carrier, which is non-toxic to the cells and the individual. Such carrier may be the growth medium in which the cells were grown, or any suitable buffering medium such as phosphate buffered saline. The cells may be administered alone or as an adjunct therapy in conjunction with other therapeutics known in the art for the treatment or prevention of unwanted immune responses for example but not limited to glucocorticoids, methotrexate, D-penicillamine, hydroxychloroquine, gold salts, sulfasalazine, TNFα or interleukin-1 inhibitors, and/or other forms of specific immunotherapy.

4. Compositions

[0120] The present invention also contemplates immunomodulating compositions, including vaccines, comprising the lectin-binding agent as described in Section 3.1 and an antigen as described in Sections 3.2 (therapeutic/prophylactic agents) as active ingredients for the treatment or prophylaxis of various diseases or conditions associated with the presence or aberrant expression of a target antigen. These therapeutic/prophylactic agents can be administered to a patient either by themselves, or in compositions where they are mixed with a suitable pharmaceutically acceptable carrier and/or diluent, or an adjuvant.

[0121] The components of the compositions of the invention may be used as active ingredients in the preparation of pharmaceutical compositions and/or vaccines. Such preparation uses routine methods known to persons skilled in the art. Typically, such

compositions and vaccines are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The active immunogenic ingredients are often mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, phosphate buffered saline, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants that enhance the effectiveness of the vaccine. Examples of adjuvants which may be effective include but are not limited to: surface active substances such as hexadecylamine, octadecylamine, octadecyl amino acid esters, lysolecithin, dimethyldioctadecylammonium bromide, N, N-dicoctadecyl-N', N'bis(2-hydroxyethyl-propanediamine), methoxyhexadecylglycerol, and pluronic polyols; polyamines such as pyran, dextransulfate, poly IC carbopol; mineral gels such as aluminum phosphate, aluminium hydroxide or alum; peptides such as muramyl dipeptide and derivatives such as N-acetyl-muramyl-L-threonyl-D-isoglutamine (thur-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 1983A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, dimethylglycine, tuftsin; oil emulsions; trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion; lymphokines; QuilA and immune stimulating complexes (ISCOMS). For example, the effectiveness of an adjuvant may be determined by measuring the amount of antibodies resulting from the administration of the vaccine, wherein those antibodies are directed against one or more antigens presented by the treated cells of the vaccine.

[0122] The active ingredients should be administered in a pharmaceutically acceptable carrier, which is non-toxic to the cells and the individual. Such carrier may be the growth medium in which the cells were grown. Compatible excipients include isotonic saline, with or without a physiologically compatible buffer like phosphate or Hepes and nutrients such as dextrose, physiologically compatible ions, or amino acids, and various culture media suitable for use with cell populations, particularly those devoid of other immunogenic components. Carrying reagents, such as albumin and blood plasma fractions and nonactive thickening agents, may also be used. Non-active biological components, to the extent that

they are present in the vaccine, are preferably derived from a syngeneic animal or human as that to be treated, and are even more preferably obtained previously from the subject. The injection site may be subcutaneous, intraperitoneal, intramuscular, intradermal, or intravenous.

[0123] If soluble actives are employed, the soluble active ingredients can be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic basis such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic basis as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

[0124] If desired, devices or pharmaceutical compositions or compositions containing the vaccine and suitable for sustained or intermittent release could be, in effect, implanted in the body or topically applied thereto for the relatively slow release of such materials into the body.

[0125] Techniques for formulation and administration may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition. Suitable routes may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

[0126] The dosage to be administered may depend on the subject to be treated inclusive of the age, sex, weight and general health condition thereof. In this regard, precise amounts of the agent(s) for administration will depend on the judgement of the practitioner. In determining the effective amount of the agent(s) to be administered in the treatment of a disease or condition, the physician may evaluate the progression of the disease or condition over time. In any event, those of skill in the art may readily determine suitable dosages of the agents of the invention without undue experimentation. Cell-containing compositions and vaccines are suitably administered to a patient in the range of between about 10^4 and 10^{10}, and more preferably between about 10^6 and 10^8 treated cells/administration. The dosage of the actives administered to a patient should be sufficient to effect a beneficial response in the patient over time such as a reduction in the

symptoms associated with the cancer or tumour. Usual patient dosages for systemic administration range from 1-2000 mg/day, commonly from 1-250 mg/day, and typically from 10-150 mg/day. Stated in terms of patient body weight, usual dosages range from 0.02-25 mg/kg/day, commonly from 0.02-3 mg/kg/day, typically from 0.2-1.5 mg/kg/day.

5. *Methods for modulating immune responses*

[0127] The compositions of the invention may be used for modulating an immune response in a subject. Thus, in one embodiment there is provided a method for enhancing an immune response in a subject by administering to the subject the compositions or vaccines of the invention. Advantageously, the immune response is a cell-mediated immune response (e.g., a T-cell mediated response, which desirably includes CD4+ and/or CD8+T cells).

[0128] The active ingredients of the compositions may be administered either sequentially, simultaneously or separately.

[0129] Also encapsulated by the present invention is a method for treatment and/or prophylaxis of a disease or condition, comprising administering to a patient in need of such treatment an effective amount of a composition as broadly described above. In certain embodiments, the composition is designed to stimulate or augment an immune response to a target antigen. In these embodiments, the target antigen is typically associated with or responsible for a disease or condition which is suitably selected from cancers, infectious diseases and diseases characterised by immunodeficiency. Examples of cancer include but are not limited to ABL1 protooncogene, AIDS related cancers, acoustic neuroma, acute lymphocytic leukaemia, acute myeloid leukaemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, anal cancer, angiosarcoma, aplastic anaemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumours, breast cancer, CNS tumours, carcinoid tumours, cervical cancer, childhood brain tumours, childhood cancer, childhood leukaemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, colorectal cancers, cutaneous T-cell lymphoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumour, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anaemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers,

gastrointestinal-carcinoid-tumour, genitourinary cancers, germ cell tumours, gestational-trophoblastic-disease, glioma, gynaecological cancers, haematological malignancies, hairy cell leukaemia, head and neck cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, hodgkin's disease, human papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer, intraocular melanoma, islet cell cancer, kaposi's sarcoma, kidney cancer, langerhan's-cell-histiocytosis, laryngeal cancer, leiomyosarcoma, leukaemia, li-fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid-tumour-of-kidney, medulloblastoma, melanoma, merkel cell cancer, mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders, nasal cancer, nasopharyngeal cancer, nephroblastoma, neuroblastoma, neurofibromatosis, nijmegen breakage syndrome, non-melanoma skin cancer, non-small-cell-lung-cancer-(NSCLC), ocular cancers, oesophageal cancer, oral cavity cancer, oropharynx cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer, paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral-neuroectodermal-tumours, pituitary cancer, polycythemia vera, prostate cancer, rare-cancers-and-associated-disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, rothmund-thomson syndrome, salivary gland cancer, sarcoma, schwannoma, sezary syndrome, skin cancer, small cell lung cancer (SCLC), small intestine cancer, soft tissue sarcoma, spinal cord tumours, squamous-cell-carcinoma-(skin), stomach cancer, synovial sarcoma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis-/-ureter), trophoblastic cancer, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer, vulva cancer, Waldenstrom's-macroglobulinemia, Wilms' tumour.

[0130] In other embodiments, the composition of the invention could also be used for generating large numbers of $CD8^+$ or CD4+ CTL, for adoptive transfer to immunodeficient individuals who are unable to mount normal immune responses. For example, antigen-specific $CD8^+$ CTL can be adoptively transferred for therapeutic purposes in individuals afflicted with HIV infection (Koup *et al.*, 1991, *J. Exp. Med.* **174**: 1593-1600; Carmichael *et al.*, 1993, *J. Exp. Med.* **177**: 249-256; and Johnson *et al.*, 1992, *J. Exp. Med.* **175**: 961-971), malaria (Hill *et al.*, 1992, *Nature* **360**: 434-439) and malignant tumours such as melanoma (Van der Brogen *et al.*, 1991, *Science* **254**: 1643-1647; and Young and Steinman 1990, *J. Exp. Med.*, **171**: 1315-1332).

[0131] In other embodiments, the composition is suitable for treatment or prophylaxis of a viral, bacterial or parasitic infection. Viral infections contemplated by the present invention include, but are not restricted to, infections caused by HIV, Hepatitis, Influenza, Japanese encephalitis virus, Epstein-Barr virus and respiratory syncytial virus. Bacterial infections include, but are not restricted to, those caused by *Neisseria* species, *Meningococcal* species, *Haemophilus* species *Salmonella* species, *Streptococcal* species, *Legionella* species and *Mycobacterium* species. Parasitic infections encompassed by the invention include, but are not restricted to, those caused by *Plasmodium* species, *Schistosoma* species, *Leishmania* species, *Trypanosoma* species, *Toxoplasma* species and *Giardia* species.

[0132] In still other embodiments, the composition is designed to induce tolerance or otherwise attenuate an immune response to a target antigen. In these embodiments, the target antigen is typically associated with or responsible for a disease or condition which is suitably selected from transplant rejection, graft *versus* host disease, allergies, parasitic diseases, inflammatory diseases and autoimmune diseases. Examples of transplant rejection, which can be treated or prevented in accordance with the present invention, include rejections associated with transplantations bone marrow and of organs such as heart, liver, pancreas, kidney, lung, eye, skin etc. Examples of allergies include asthma, hayfever, food allergies, animal allergies, atopic dermatitis, rhinitis, allergies to insects, fish, latex allergies etc. Autoimmune diseases that can be treated or prevented by the present invention include, for example, psoriasis, systemic lupus erythematosus, myasthenia gravis, stiff-man syndrome, thyroiditis, Sydenham chorea, rheumatoid arthritis, diabetes and multiple sclerosis. Examples of inflammatory disease include Crohn's disease, colitis, chronic inflammatory eye diseases, chronic inflammatory lung diseases and chronic inflammatory liver diseases.

[0133] The effectiveness of the immunization or tolerance may be assessed using any suitable technique. For example, CTL lysis assays may be employed using stimulated splenocytes or peripheral blood mononuclear cells (PBMC) on peptide coated or recombinant virus infected cells using ^{51}Cr or Alamar Blue™ labeled target cells. Such assays can be performed using for example primate, mouse or human cells (Allen *et al.*, 2000, *J. Immunol.* 164(9): 4968-4978 also Woodberry *et al.*, *infra*). Alternatively, the efficacy of the immunization may be monitored using one or more techniques including, but not limited to, HLA class I tetramer staining - of both fresh and stimulated PBMCs (see

for example Allen *et al.*, *supra*), proliferation assays (Allen *et al.*, *supra*), ELISPOT assays and intracellular IFN-gamma staining (Allen *et al.*, *supra*), ELISA Assays - for linear B cell responses; and Western blots of cell sample expressing the synthetic polynucleotides

[0134] In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

EXAMPLES

EXAMPLE 1

Use of lactulose to improve the efficacy of an anti-cancer vaccine in a mouse model

[0135] C57bl/6j mice were aged and sex matched The mice were given a vaccine comprising B16F10-B7.1 high treated with IFN-gamma and IFN-beta, as described in International Publication No. WO 01/88097, and the cells were irradiated for 1.5 hours at 5×10^7 cells/ml. Mice received either vaccine cells alone or including an addition of 20 mg/ mL solution of lactulose at 1:1 ratio to the cell suspension. The vaccine was mixed well and injected as final volume of 400 µL intraperitoneally per mouse. This resulted in a vaccine dose of 1×10^7 cells per injection including a final 10 mg/mL lactulose per vaccination in cohort (n=4) of mice receiving vaccine plus lactulose. Each mouse was vaccinated with the above cell mix per week, over a six week period. On the seventh week they were challenged with 5×10^5 live B16F10-B7.1med cells (containing no lactulose), subcutaneously on the rear.

[0136] During the course of this experiment, a second group (no lactulose) were vaccinated weekly over a five week period but with a irradiated (1.5 hours) B16F10B7.1high + IFNγβ at a total 1×10^7 cells in a 200 µL injection, intraperitoneally per mouse. On the sixth week, the second group also received the challenge with 5×10^5 live cells of B16F10-B7.med (no lactulose) subcutaneously. The third group of 3 control mice received no vaccine before the challenge and as usual all succumbed to the challenge with 5×10^5 live cells of B16F10-B7.1med subcutaneously. Figure 1 shows there was a large improvement in this experiment in the efficacy of the vaccine given to treated mice when lactulose was included (100 percent of the mice survived) versus the vaccine alone cohort which all succumbed except for 4 out of 25 mice.

[0137] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

[0138] The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also

includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

DATED this 16 October, 2003

MONASH UNIVERSITY AND GRIFFITH UNIVERISTY

By their Patent Attorneys

DAVIES COLLISON CAVE

BIBLIOGRAPHY

1. Maher, S. *et al., Immunol Cell Biol* 2002, **80**:131-137.
2. Nagata and Golstein, 1995
3. Kabelitz and Janssen, 1997
4. Bussing *et al., Cancer Lett* 1996; **99**: 59-72.
5. Bussing *et al., Cancer Lett* 1988; **130**: 57-68.
6. Rabinovich *et al.*, 2002, *Trends Immunol.* **23**:313-320;
7. Rabinovich *et al., J. Leuk Biol* 2002, **71**:741-752.
8. Blaser C. *et al., Euro. J Immunol,* 1998, **28.**
9. Rabinovich *et al., J. Leuk Biol.,* 2002, **71**:741-752.
10. Perillo, NL *et al., Nature,* 1995, **378**:76-???.
11. Pace *et al., J. Immunol* 2000, **165**:2331-2334.
12. Nguyen JT *et al.,* J Immunol 2001: **167**:5697-5707.
13. Carlow DA *et al., J. Immunol.,* 2001, **167**:6841-6848.
14. Tsobi and Fukuda, Bioessays 2001; **23**:46-53.
15. Blessing JJ *et al., Cell Immunol.,* 2001, **213**:72-81.
16. Cyster *et al., EMBO J.* 1991; **10**:893-902.
17. Ardman *et al., Proc. Natl. Acad. Sci. USA* 1992; **89**: 5001.
18. Manjunath *et al., Nature* 1995; **377**:535-5380.
19. Priatel *et al., Immunity* 2000; **12**:273-283.
20. Brown *et al., J. Biol. Chem.* 1996; **271**:27686-27695.
21. Brewer, *Biochmica Biophys Acta,* 2002; **1572**:255-262.
22. Xu and Weiss, 2002.
23. Provinciali M. *et al* (1992), *J. Immunol. Meth.* **155**: 19-24.
24. Vollenweider, I. And Groseurth, P. J. (1992, *J. Immunol. Meth.* **149**: 133-135)
25. Loeffler, D. A., *et al.* (1992, *Cytom.* **13**: 169-174).

26. Rivoltini, L., *et al.* (1992, *Can. Immunol. Immunother.* **34**: 241-251).

27. Chang, A. E. et al (1993, *Cancer Res.* **53**: 1043-1050).

28. Deveraux *et al.* 1984, *Nucleic Acids Research* **12**, 387-395.

29. Freeman *et al.* (1989, *J. Immunol.* **143**: 2714-2722).

30. Freeman *et al.* (1992, *Blood* **79**: 489-494), and in the GenBank database under locus designations HUMIGB7 (Accession number M27533) and NM_005191 (Accession number NM_005191).

31. Azuma *et al.* (1993, *Nature* **336**: 76-79)

32. Chen *et al.* (1994, *J. Immunol.* **152**: 4929-4963), and in the GenBank database under locus designation NM_006889 (Accession number NM_006889).

33. McHugh *et al.* (1998, *Clin. Immunol. Immunopathol.* **87**(1): 50-59).

34. Faas *et al.* (2000, *J. Immunol.* **164**(12): 6340-6348).

35. Jeannin *et al.* (2000, *Immunity* **13**(3): 303-312).

Sturmhoefel *et al.* (1999, *Cancer Res.* **59**: 4964-4972).

36. Chapman *et al* (1999, *Nature Biotechnology* **17**: 780-783).

37. Pel *et al* (1995, *Immunol Rev* **145**: 229-50).

38. Itoh *et al* (1994, *J. Immunol.* **153**: 1202-1215).

39. Cole *et al* (1995, *Cancer Res.* **55**: 748-752).

40. Koup *et al.*, 1991, *J. Exp. Med.* **174**: 1593-1600.

41. Carmichael *et al.*, 1993, *J. Exp. Med.* **177**: 249-256.

42. Johnson *et al.*, 1992, *J. Exp. Med.* **175**: 961-971.

43. Hill *et al.*, 1992, *Nature* **360**: 434-439.

44. Van der Brogen *et al.*, 1991, *Science* **254**: 1643-1647.

45. Young and Steinman 1990, *J. Exp. Med.*, **171**: 1315-1332.

46. Allen *et al.*, 2000, *J. Immunol.* **164**(9): 4968-4978

47. Woodberry *et al.*, *infra.*

48. Clevenger, *et al* (1987, *J Cell Physiol.* **130**: 336-343).

49. Kuhar and Lehman (1991, *Oncogene* **6**: 1499-1506).

50. Fodor *et al.*, (1991, *Science* **251**:767-777).

51. Kazal *et al.*, (1996, *Nature Medicine* **2**:753-759).

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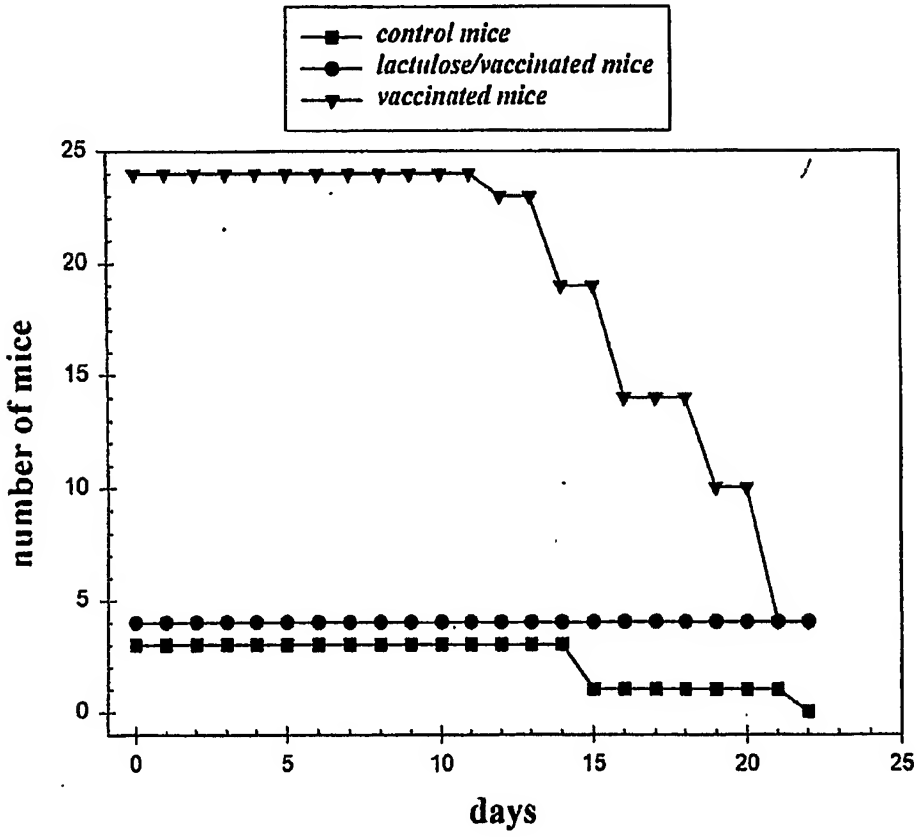
FIGURE 1

C57bl/6j mice
vaccinated with 6 weekly injections of [irradiated B16B7.1High + IFNg(72)b(48)]
challenged on 18.6.2002 with B16F10 live cells



Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/AU04/001429

International filing date: 18 October 2004 (18.10.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/511,745
Filing date: 16 October 2003 (16.10.2003)

Date of receipt at the International Bureau: 16 November 2004 (16.11.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse